[CONTRIBUTION FROM PULP MILLS RESEARCH AND THE DEPARTMENTS OF CHEMISTRY AND CHEMICAL ENGINEERING, UNIVERSITY OF WASHINGTON]

Lignin. VI. Molecular Weights of Lignin Sulfonates by Light Scattering[1]

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Western hemlock wood has been treated with a sodium bisulfite-sulfurous acid solution to obtain the lignin sulfonates in aqueous solution. This solution was exhaustively dialyzed and the non-dialyzable lignin sulfonates were recovered and separated into a number of fractions by a reprecipitation method. The lignin sulfonate fractions were characterized by ultraviolet absorption spectra and absorptivities, and by diffusion coefficients. Light scattering measurements were carried out on some of the lignin sulfonates and from the turbidity values found, weight average molecular weights were estimated. The relationships found to exist between the molecular weights and the diffusion coefficients are explained by considering that lignin molecules exist in solution as non-rigid chains.

Introduction

Several studies previously have been made of the molecular weight of various lignins[3] and a report has been submitted from this Laboratory[4] showing that non-dialyzable lignin sulfonates, after fractionation, are of approximately constant composition and manifest progressively different diffusion coefficients and hence are presumably of different molecular weights as expected for a polymeric series of lignins. Although recent work has been conducted by Gralen,[5] Ritter[6,7] and Ivarsson[8] on the molecular weight of lignins, the present investigation was carried out using light scattering and other methods to extend these results by measuring the molecular weights and frictional coefficients of several purified lignin sulfonates which had been more extensively fractionated.

Experimental

Preparation of Non-dialyzable Lignin Sulfonates.—The lignin sulfonates were secured from a Western Hemlock (*Tsuga heterophylla*) tree cut by the authors, then debarked and deknotted. Bole wood was converted by use of a table saw to wood meal (10 to 35 mesh) which was air-dried and placed in a large glass column and extracted exhaustively by continuous percolation with a solution 50 volume per cent. each in ethanol and benzene whereby 1.7 weight per cent. of the air dry wood was removed as extractives. The wood meal was then extracted batchwise and nearly exhaustively with water at 80° whereby an additional 1.1% of the air-dried wood meal was extracted. This extracted wood meal was air-dried and analyzed with the following results on the oven-dry basis: 6.14% H_2O and 5.24% OCH_3.

Delignification nearly to completion was carried out by sealing in several glass bombs a total of 125 g. of air-dried extracted wood meal together with 1000 ml. of an aqueous sulfurous acid–sodium bisulfite solution (50 g. SO_2/liter and 9.67 g. Na_2O/liter, or 4% "free SO_2" and 1% "combined SO_2") heating for six and a half hours at 135°, then cooling and opening the bombs. The contents of the bombs were combined and the cellulosic residue was filtered off, washed with distilled water, air-dried, weighed and found to be 44% of the air-dried wood meal.

The filtrate and washings were combined, concentrated to 600 ml. by vacuum evaporation and then exhaustively dialyzed against distilled water. Dialysis was conducted by use of Visking cellophane dialyzing tube (wall thickness, 0.0044 cm.; diameter, 4.13 cm.; length, 100 cm.) which was placed in a 4.8-cm. diameter Pyrex tube. The solution to be dialyzed was placed inside the cellophane tube and distilled water was flowed into the outer chamber at a rate of about 5 ml. per minute with an outer chamber liquid level of 30 cm. maintained by means of an overflow tube. Contents of the inner and outer chambers were mixed by bubbling nitrogen gas through the liquids. Dialysis was continued for 120 hours at which time the ratio of the ultraviolet light absorbance at 2800 Å. of the overflow solution to the solution inside the membrane was about 0.007. Of the initial material absorbing 2800 Å. ultraviolet radiation, about 50% was dialyzed through the membrane together with sugars, sugar sulfonates, aldonic acids and inorganic substances.

The non-dialyzable sodium lignin sulfonate solution was concentrated under vacuum to 150 ml., de-ashed by use of cation-exchange resin and then extracted with three equal volumes of ether. The extracted lignin sulfonic acids were neutralized with standard sodium hydroxide and 2.07 meq. strong acid/g. total solids were found present. This sodium lignin sulfonate solution was concentrated by vacuum evaporation to 105 ml. and analyzed[4] with the following results: 150.0 g. total solids/liter; 2.20 g. reducing substance calculated as glucose/liter; absorptivity, a_a = 12.6 liters/g. cm. at 2800 Å.; diffusion coefficient, D = (11.6) (10^{-7}) cm.²/sec. at 25.2° in 0.02 N NaCl solution.

Fractionation Procedure.—A 100-ml. aliquot of the sodium lignin sulfonate solution was placed in a liter bottle together with sufficient solid NaCl to make 800 ml. 0.1 M in NaCl. An ethanol solution (700 ml. at 95% by volume) was added dropwise with vigorous stirring and over a 20-minute period while the precipitation bottle was situated in a water-bath at 25°. After stirring for an additional five minutes, the solution was poured off into four centrifuge bottles and centrifuged for five minutes. The clear solution of 766 ml. volume was poured out of the centrifuge bottles and called fraction 1. Since the total liquid phase amounted to about 792 ml., only about 3.3% of the light phase remained with the heavy phase which was present mostly as a soft gum on the sides and on the bottom of the bottle. The heavy phase was redissolved in 74 ml. of water and 40 ml. of 2 M NaCl. Then 684 ml. of 95% ethanol was added as before and the solution resulting was called fraction 2. This procedure of redissolving and reprecipitating with progressively lower concentrations of alcohol was continued until ten fractions were secured and the results are given in Table I. The aqueous solution of the solutes insoluble at 69.5% ethanol by volume was taken as the final fraction 10. After fraction 3 had been separated, the heavy phase could not be entirely redissolved in water. Perhaps one-tenth of 1% of the solutes remained behind as flocculent particles and these were not removed but were carried through the remaining steps of the fractionation. Ethanol was evaporated from each fraction, which was adjusted to a volume of 100 ml., and then saturated with toluene and stored in a refrigerator. When examined further, the toluene was removed.

Characterization of Fractions.—The concentration of total non-volatile solids in each fraction was determined by drying an aliquot for 40 hours under vacuum at 60° and results are given in Table I. Chloride was estimated by the Mohr method and the concentration of sodium lignin sulfonates was obtained by subtracting the sodium chloride from the

(1) Presented in part at the Pacific Northwest Regional Meetings of the American Chemical Society at Corvallis, Oregon, in June, 1952, and at Moscow, Idaho, in June, 1953.

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TABLE I

FRACTIONATION OF NON-DIALYZABLE SODIUM LIGNIN SULFONATES

Fraction no.	Ethanol concn., % by vol.	NaCl concn., M	NaLS[a] concn., g./l.	Cumulative U.V. abs.,[b] %	Cumulative NaLS wt., %	Absorptivity ratios[c] A	Absorptivity ratios[c] B	a,[d] l./g. cm.	D,[e] cm.²/sec. × 10^7
1	82.5	0.570	19.44	12.0	13.0	1.47	3.48	11.7	22.0
2	80.5	1.009	11.24	19.9	20.5	1.46	3.52	13.2	19.1
3	78.5	0.809	21.62	33.4	34.9	1.41	3.30	11.8	16.0
4	76.5	.799	15.56	43.4	45.2	1.40	3.25	12.1	14.7
5	74.5	.803	18.34	55.2	57.5	1.39	3.16	12.1	12.4
6	72.5	.801	25.18	72.0	74.3	1.37	3.07	12.5	9.6
7	71.5	.800	18.76	84.3	86.8	1.34	2.99	12.3	8.0
8	70.5	.800	8.32	90.1	92.3	1.34	2.96	13.2	5.9
9	69.5	.799	0.44	96.5	98.6	1.31	2.81	12.7	4.4
10	0	.005	4.06	99.3	101.3	1.24	2.59	12.6	2.7

[a] NaLS = sodium lignin sulfonate. This concentration was calculated from the difference between the total solids and the chloride determinations. [b] Ultraviolet absorption was measured at 2800 Å. [c] Absorptivity ratios A and B are ratios of absorbance at 2800 Å. to that at about 2600 Å., and of absorbance at 2800 Å. to that at 3100 Å., respectively. [d] a = absorptivity at 2800 Å. [e] D = diffusion coefficient in 0.02 M aqueous NaCl at 25.2°.

total solids. A modification of the Munson and Walker[9] procedure was used to determine the concentration of reducing groups in five of the fractions. Specific volumes were evaluated by the technique and calculation method recommended by Svedberg and Pedersen[10] and some results are given in Table II. Ultraviolet absorption spectra were determined for the fractions in 0.004 M aqueous sodium chloride solution at 50 mg. sodium lignin sulfonate/liter from 2500 to 3100 Å. using a Beckman model DU spectrophotometer. From the results at 2800 Å., absorptivities, a_a, for the sodium lignin sulfonates were computed by the relationship given in the list of symbols and in this regard our notation now conforms to that recommended by the Joint Committee on Nomenclature in Applied Spectroscopy.[11]

TABLE II

SPECIFIC VOLUME[a] OF SODIUM LIGNIN SULFONATES[b] IN KCl

0.02 M KCl soln. NaLS, g./l.	0.02 M KCl soln. V_{NaLS}, ml./g.	0.20 M KCl soln. NaLS, g./l.	0.20 M KCl soln. V_{NaLS}, ml./g.	0.997 M KCl soln. NaLS, g./l.	0.997 M KCl soln. V_{NaLS}, ml./g.
9.2	0.611_1	9.1	0.619	7.3	0.613
18.3	$.614_4$	19.1	$.622_2$	15.4	.615

[a] 25.2°. [b] Previously prepared non-dialyzable lignin sulfonates of $D = 7.4 \times 10^{-7}$ cm.²/sec.

NOTATION

A_s = $\log_{10} 1/T$ = absorbance, where T is the ratio of the transmittance of the solution to that of the solvent

a = $\frac{1}{1000cl} \log_{10} 1/T$ = absorptivity, l./g. cm.

a = major axis of prolate spheroid, Å.

b = minor axis of prolate spheroid, Å.

c = sodium lignin sulfonate concn., g./ml.

D = obsd. diffusion coefficient, cm.²/sec.

D_0 = diffusion coefficient at infinite diln., cm.²/sec.

D_s = diffusion coefficient for spherical molecule of equal vol. as molecules of diffusion coefficient D, cm.²/sec.

f = obsd. mol. frictional coefficient = kT/D

f_0 = mol. frictional coefficient at infinite diln.

f_s = mol. friction coefficient for spherical molecules of equal vol. as molecules of mol. friction coefficient, f

H = $\frac{32\pi^3 n (dn/dc)^2}{3\lambda^4 N}$ = light scattering constant

I = intensity of radiation, with no subscripts for unpolarized light, with superscripts V or H for vertical or horizontal components of the light, respectively; and with subscripts i, s, f or t for incident, scattered, fluorescent or transmitted light, respectively

k = Boltzmann constant

K = light scattering cell constant relating the apparent scattering ratio to turbidity as shown in eq. 2

l = path length of light beam through a soln., cm.

M = mol. wt.

n = refractive index of soln.

n_0 = refractive index of solvent

N = Avogadro's number

R = universal gas constant

R^V = $\frac{I_s^V + I_f^V}{I_t^V}$ = apparent vertical transverse scattering ratio

R^H = $\frac{I_s^H + I_f^H}{I_t^H}$ = apparent horizontal transverse scattering ratio

T = absolute temperature, °K.

V_{NaLS} = specific volume of lignin sulfonates, ml./g.

η_0 = viscosity of solvent, g./cm. sec.

$[\eta]$ = intrinsic viscosity, 100 ml./g.

λ = wave length of light, Å.

ρ_f = depolarization factor for fluorescent light = I_f^H/I_f^V

ρ_s = depolarization factor for unpolarized primary light = I_s^H/I_s^V

τ = $\frac{1}{l} \log e \frac{I_s}{I_t}$ = turbidity, cm.$^{-1}$

Diffusion coefficients were determined using a previously described solution-to-gel method[12] wherein diffusion proceeded at 25.2° into an agar gel from an aqueous solution containing sodium lignin sulfonates at about 250 mg./liter and also 0.02 M NaCl. The gel was made using purified agar at 0.37% in 0.02 M NaCl and was contained in a cell made from silica microscope slides through which 2800 Å. ultraviolet radiation was passed for observation of absorption at various distances from the boundary. Graphs of this distance *versus* the ratio of position concentration to boundary concentration on a "probability" scale proved to be nearly straight lines for the fractions. The absorbances were determined at 2800 Å. for the solutions from which the diffusion took place and these values were compared with the absorbancies estimated for the boundary by extrapolation of the observations made on the gel phase. The ratios obtained were 0.99, 0.98, 0.96, 0.98, 0.97 and 0.88 and 0.89 for fractions 4 through 10, respectively, and some hindrance is indicated to diffusion of fractions 9 and 10. Diffusion coefficients were also measured for some pure phenols of known molecular weight.

Light Scattering.—Measurements were made using one centimeter square "Corex" Beckman cells (carefully finished and polished on four sides by the Beckman Company, Pasadena, California) in a Brice–Phoenix Light Scattering Photometer,[13] Phoenix Precision Instrument Company, Philadelphia, Penna. A special cell holder was built using a design kindly suggested by Dr. Walter Dandliker. The holder consisted of a square platform on top of which the

(9) L. S. Munson and P. H. Walker, THIS JOURNAL, **28**, 663 (1906).

(10) T. Svedberg and K. O. Pedersen, "The Ultracentrifuge," Clarendon Press, Oxford, 1940, p. 58.

(11) Report No. 6, *Anal. Chem.*, **24**, 1349 (1952).

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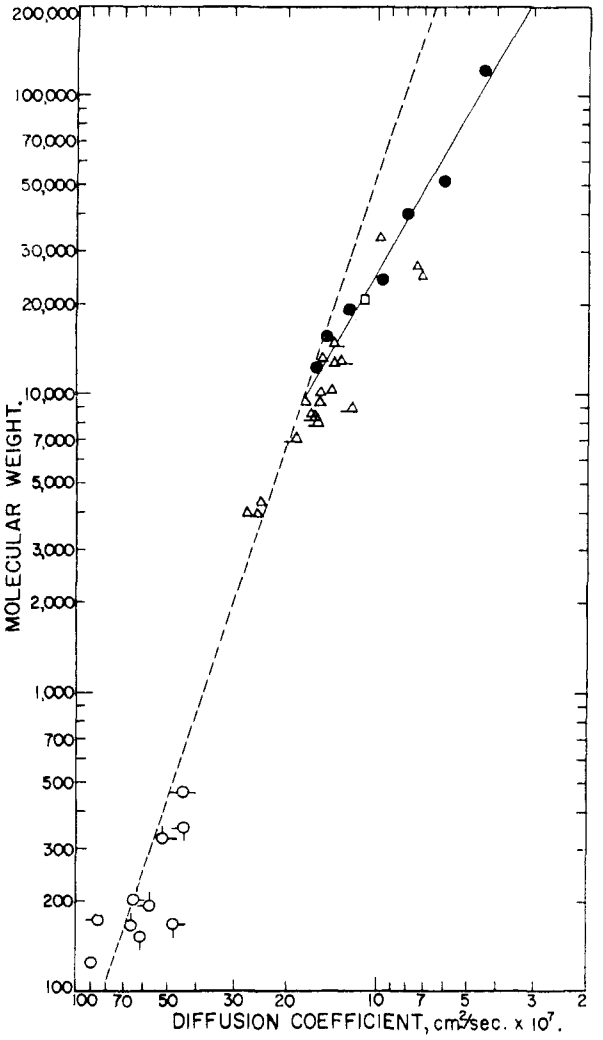

Fig. 1.—Molecular weight-diffusion coefficient relationship for lignin preparations and other substances: △, thioglycolic acid lignin (Gralen); _△, hypobromite lignin, (Gralen); △_, acetic acid lignin (Gralen); □, ammonium lignin sulfonate (Ritter, *et al.*); ●, sodium lignin sulfonate fractions (authors); ◊, acetovanillone; -o-, azogrenadin S; -♀, conidendrin; -◊, ferulic acid; O, guaiacol; -o, Na *p*-phenolsulfonate; ◊-, orange II; o-, tryptophan; ♀-, vanillic acid; ♀, vanillin.

cell is held centered by means of slotted walls. By use of additional slits, the optical system of the instrument was adapted to the size of the cell. The width of the primary beam was reduced to about 3.5 mm. An unpolarized primary beam was used in all measurements. The "Polaroid" analyzer supplied with the instrument was used to determine separately the vertical and horizontal components of the transmitted and the scattered light. The opal glass depolarizer usually found in the photomultiplier tube housing, was removed to increase sensitivity. The resulting slight difference in sensitivity of the response of the photomultiplier tube to the vertical and horizontal electric vector (in this case less than 1%) was immaterial since in all observations the ratios of intensities of either the vertical or the horizontal components of the scattered and transmitted light were measured.

The instrument was calibrated against a du Pont colloidal silica solution called "Ludox," the turbidity of which was determined by transmission measurements using a Beckman spectrophotometer,[14,15] extrapolating the results to an infinitely small slit. The turbidity of the "Ludox" solution used for calibration of the cells was found to be 0.0699 cm.$^{-1}$ for the blue light and 0.0279 cm.$^{-1}$ for the green light. The solution used was cleaned by centrifuging at about 6,000 r.p.m. for one hour. The values of the cell constant were found to be K = 0.556 and 0.475 cm.$^{-1}$ for the 5461 Å. and 4358 Å. radiations, respectively. Refractive index differences were measured in a Brice–Phoenix Differential Refractometer.[16]

Seven lignin sulfonate fractions were studied using the light scattering procedure. Sufficient sodium chloride was added to the sodium lignin sulfonate solutions to bring them to 1.0 M in NaCl, *i.e.*, an ionic strength of 1.0. This was considered to provide sufficient screening of the charges on the lignin polymer[17] since in ultracentrifuge studies in this Laboratory it has been found that substantially constant values of the sedimentation velocity constant were obtained at ionic strengths above 0.1. To remove dust, each master lignin sulfonate solution was first filtered with a slight positive pressure through a Corning sintered glass Ultra Fine filter and solutions of the desired lignin concentration were obtained by dilution of this filtered master solution with 1.0 M NaCl solution filtered in the same manner. Before use, all solutions were centrifuged for two hours at 20,000 r.p.m. in a refrigerated International centrifuge equipped with a high speed attachment, and were then pipetted into clean cells for light scattering observations. Reproducible results were secured with solutions clarified by centrifuging alone, but in most cases filtration further decreased the turbidity of the solutions. Therefore, both methods were applied. The cells and other glassware were kept scrupulously clean. After careful washing, they were rinsed with fresh doubly distilled water, kept in and dispensed directly from a flask attached to an all-glass still, and then they were finally rinsed with acetone vapor.

Because the sodium lignin sulfonate solutions showed both fluorescence and light absorption as well as light scattering, the measurements recommended by Brice, Nutting and Halwer[18] were taken instead of the usual ones. Since the scattered light is mostly vertically polarized, and the fluorescent light is largely depolarized, the two effects were observed by determining both the vertical and horizontal components of the light at the 90° angle. Scattering measurements were made with unpolarized incident light using both the blue (4358 Å.) and the green (5461 Å.) mercury line. The apparent transverse scattering ratios, R^H and R^V, were computed from the observed galvanometer de-

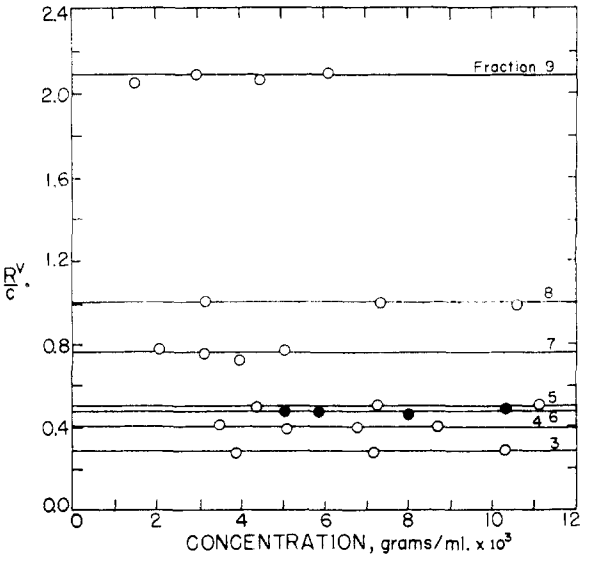

Fig. 2. - R^V/c *versus* sodium lignin sulfonate concentrations at 5461 Å.

(14) W. F. H. M. Mommaerts, *J. Coll. Sci.*, **7**, 71 (1952).
(15) A. Oth, J. Oth and V. Desreux, *J. Poly. Sci.*, **10**, 551 (1953).
(16) B. A. Brice, M. Halwer and R. Speiser, *J. Opt. Soc. Am.*, **41**, 1033 (1951).
(17) R. M. Fuoss and D. Edelson, *J. Poly. Sci.*, **6**, 787 (1951).
(18) B. A. Brice, C. C. Nutting and M. Halwer, THIS JOURNAL, **75**, 824 (1953).

flections and known filter constants. Specific values of these ratios were also calculated and Fig. 2 illustrates how these quantities varied with concentration for the vertical orientation of the electric vector with 5461 Å. radiation. By extrapolation to infinite dilution, the limiting values shown in Tables III and IV were obtained.

TABLE III

LIGHT SCATTERING BY SODIUM LIGNIN SULFONATE FRACTIONS (4358 Å.)

Fraction no.	$\left(\frac{R^V}{c}\right)_{c=0}$	$\left(\frac{R^H}{c}\right)_{c=0}$	ρ_f	$(H)\text{-}(10)^5$	$(c/\tau)_{c=0}$	$\bar{M}_w$
3	2.60	1.60	0.74	1.40	8.34	8,600
4	2.70	1.20	.72	1.25	5.26	15,200
5	3.30	1.60	.70	1.25	3.70	21,600
6	4.35	2.03	.67	1.25	3.03	26,400
7	5.15	1.80	.65	1.25	2.11	37,900
8	6.00	1.80	.63	1.25	1.64	48,800
9	9.12	1.84	.62	1.09	0.662	138,000

TABLE IV

LIGHT SCATTERING BY SODIUM LIGNIN SULFONATE FRACTIONS (5461 Å.)

Fraction no.	$\left(\frac{R^V}{c}\right)_{c=0}$	$\left(\frac{R^H}{c}\right)_{c=0}$	ρ_f	$(H)\text{-}(10)^5$	$\left(\frac{c}{\tau}\right)_{c=0}$	$\bar{M}_w$
3	0.285	0.041	0.69[a]	0.526	15.4	12,300
4	.401	.100	.67	.471	13.5	15,700
5	.500	.120	.64	.471	11.08	19,200
6	.482	.050	.60	.471	8.65	24,400
7	.763	.070	.58	.471	5.33	40,000
8	1.03	.110	.55	.471	4.04	52,600
9	2.10	.210	.52	.405	2.02	122,000

[a] Extrapolated.

The depolarization factors for fluorescence, ρ_f, were measured directly by interposing in the scattered beam "sharp cut-off" filters having essentially zero transmittance up to a wave length slightly longer than that of the primary beam. These filters were: Corning No. 3384, C. S. 3–70 for the blue primary light, and Corning No. 2424, C. S. 2–63 for the green primary light. The depolarization factor for scattering, ρ_s, was taken to be 0.03 and constant for all the lignin sulfonate fractions with both the blue and the green light. This value should be approximately correct since experiments with some higher molecular weight lignin sulfonates in the green light gave values as low as 0.03 for the R^H/R^V ratio, where

$$R^H/R^V = \frac{I_s^H + I_f^H}{I_s^V + I_f^V} \qquad (1)$$

When molecular weight increases, I_s^H and I_s^V increase while I_f^H and I_f^V are not much affected as is shown by comparison of R^H/c and R^V/c in Tables III and IV. The result, as molecular weight increases, is that R^H/R^V decreases and approaches ρ_s. For the same sample in the blue light, R^H/R^V ratio was 0.13 and by taking the rather strong fluorescence into account, it was estimated that ρ_s could not exceed and would be less than 0.05. A small error in the value taken for ρ_s will have only minor influence on the final result because of the way ρ_s enters into the computation by equation 2.

From these quantities and using the relationship given by Brice, Nutting and Halwer,[18] the specific scattering ratios or turbidities were evaluated

$$\frac{\tau}{c} = \frac{K}{2}\left[\frac{I_s^V/I_i^V}{c}\right] = \frac{K}{2}\left[\frac{R^V/c - R^H/c\rho_f}{1 - \rho_s/\rho_f}\right] \qquad (2)$$

The term $R^H/c\rho_f$ represents the contribution of fluorescence to the term R^V/c, and the former may amount to more than 80% of the latter (Table III). Thus, if the effect of fluorescence is ignored, turbidities may be greatly overestimated.

Absorption experiments with the sodium lignin sulfonate solutions in a Beckman Model DU spectrophotometer yielded the following values for the absorptivity[18]: 0.061, 0.073, 0.106 and 0.108 liter/g. cm. for the 4358 Å. radiation for the fraction Numbers 3, 6, 7 and 9, respectively. Use of the relationships of Brice and co-workers[18] indicated that corrections of about 1 or 2% should be introduced to account for absorption, but these were not applied because of their small magnitude.

Values were obtained for the ratios Hc/τ which were plotted against concentration. For the 5461 Å. measurements, these plots are linear with approximately zero slope (Fig. 3). For the 4358 Å. data, the plots are linear, but with a small negative slope varying with the absorptivity of the solution. However, absorption effects were presumably eliminated by extrapolating the results to infinite dilution to secure the limiting ratios and from these the weight average molecular weights of the lignin sulfonates were estimated by use of the Debye equation[19]

$$(Hc/\tau)_{c=0} = 1/M_w \qquad (3)$$

Results are given in Tables III and IV.

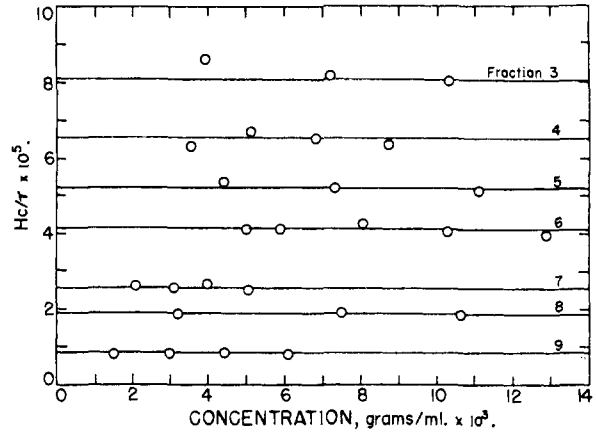

Fig. 3.—Hc/τ *versus* sodium lignin sulfonate concentrations at 5461 Å.

Discussion

The present reprecipitation method of fractionation was used in preference to our previously described procedure[4] because more nearly complete separation of light from heavy phases was readily secured, and because considerably less time was required to carry through a fractionation. The usual fractional solution procedure was not satisfactory because the precipitate obtained was a soft gum from which diffusional transfer of soluble components was very slow. Initial trials of the reprecipitation method gave rise to colloidal dispersions after one or two fractions of lower molecular weight lignin sulfonates had been removed. These were avoided by additions of sodium chloride in amount to make the final solutions about 0.1 *M* NaCl.

Close control of solvent composition was maintained and Table I shows that the fractionation was carried out between 82.5 and 69.5% ethanol concentration by volume. Cooling of a clear solution caused cloudiness and separation of the heavy phase which was redissolved by warming to a little above the original temperature. The balance and distribution of sodium lignin sulfonates in the several fractions were calculated on a weight basis (Table I) from determinations of total solids and chlorides.

The absorption spectra for 2500 to 3100 Å. radiation were obtained for the fractions and were found to be closely similar to those well known from prior work to be characteristic of lignins and lignin

(19) P. Debye, *J. App. Phys.*, **15**, 338 (1944).

sulfonates.[3,4,20] From the absorbancies observed at 2800 Å. and from the known concentrations of hemlock sodium lignin sulfonates in the fractions, absorptivities have been computed (Table I). These vary from 11.7 to 13.2 l./g. cm., show no particular trend with diffusion coefficient, and are similar to the values of 13.8, and of 12.3 l./g. cm. found at 2850 Å. for spruce calcium lignin sulfonates in water by Hägglund and Klingsted,[21] and by Stamm, Semb and Harris,[22] respectively. They are perhaps lower, possibly as a result of differences in degree of sulfonation, than the following absorptivity values which have been obtained by recalculation of the absorption coefficients previously reported[4] for the Markham, *et al.*, fractions 1, 2, 4, 7, 10 and N; 13.1, 12.7, 14.0, 13.5, 13.3 and 14.3 l./g. cm., respectively, for non-dialyzable sodium lignin sulfonates in water solution. The balance and distribution of sodium lignin sulfonates in the several fractions obtained in the present investigation were calculated on a basis of 2800 Å. radiation absorption (Table I) and these results are found to be in fairly good agreement with those calculated on a basis of weights of the sodium lignin sulfonates.

Diffusion coefficients were evaluated for the ten fractions and were found to be highest for the lignin sulfonates soluble in solutions of highest alcohol concentration and to decrease in an ordered manner for lignins soluble in solutions of increasing water content (Table I). Values reported are "D_A" averages, *i.e.*, $D_A = [\Sigma w_i d_i^{-0.5}]^{-2}$. From data for individual fractions, the calculated D_A average is 10.7×10^{-7} cm.^{2}/sec., compared with 11.6×10^{-7} cm.^{2}/sec. found for the initial unfractionated lignin sulfonates.

Molecular weights of seven of the lignin sulfonate fractions were estimated by a light scattering method described in the Experimental Part. Some light scattering characteristics of the samples are shown in Figs. 2 and 3, and the results are given in Tables III and IV. The molecular weights found by light scattering for the sodium lignin sulfonates have been plotted in Fig. 1 against the diffusion coefficients measured for these preparations. A straight correlating line has been drawn through points to provide a preliminary basis for estimation of molecular weights from diffusion coefficients of lignin sulfonates.

To aid in understanding the significance of this observed relationship, the specific volumes of non-dialyzable sodium lignin sulfonates available from previous studies[4] were determined at 25.2° at two lignin sulfonate concentrations and in 0.02, 0.20 and 0.997 *M* KCl solutions (Table II). Since the specific volume was about constant at 0.61 ml. per gram for all of these conditions, measurements were only made on the present fraction 6 at 25.2° in 1.0 *M* NaCl and the specific volume of this sodium lignin sulfonate was found to be 0.69. These results may be compared with values of 0.64 to 0.66 for hypobromite lignin, and of 0.72 and 0.74 for thioglycolic acid lignin, by Gralen,[5] 0.67 for ammonium lignin sulfonates at 25° in 1 *N* ammonium acetate solution by Ritter, *et al.*,[7] and 0.74 for calcium lignin sulfonates at 20.6° in 0.05 *N* KCl solution by Ivarsson.[8]

If the effective hydrodynamic volume of lignin sulfonate molecules in solution is approximated by a sphere not penetrated by solvent, and if the specific volume of the hydrodynamic unit is that of the polymer molecule, 0.69 ml./g., then the relationship between molecular weight and diffusion coefficient should be that expressed by the Einstein–Stokes equation

$$\text{mol. wt.} = \left(\frac{4\pi N}{3V}\right)\left(\frac{RT}{6\pi\eta_0 ND}\right)^3 = \frac{54.1 \times 10^{-15}}{D^3} \quad (4)$$

where the value of the combined constants is given for diffusion at 25.2° in an aqueous 0.02 *M* NaCl solution of viscosity 0.00892 g./cm. sec. The function is shown as a dotted line in Fig. 1 and intersects the correlating line at a molecular weight of about 10,000. This rigid sphere line also falls among the points shown representing the experimentally determined diffusion coefficients for certain known pure substances of which some are structurally related to lignin but probably of different specific volumes.

However, for lignin sulfonates of molecular weight greater than about 10,000, a substantial departure of the correlating line from the rigid sphere line is evident. In recent studies on the molecular weights of lignins, Gralen[5] and Ritter[7] also observed departures from rigid sphere behavior and explained these by assuming the hydrodynamic unit to be a rigid prolate spheroid instead of a sphere. With these assumptions, the following relationship, derived by Herzog,[23] and Perrin,[24]

$$\frac{f_s}{f} = \frac{D}{D_s} = \left[\frac{(b/a)^{2/3}}{1 - \left(\frac{b}{a}\right)^2}\right] \log_e \frac{1 + \sqrt{1 - \left(\frac{b}{a}\right)^2}}{b/a} \quad (5)$$

was used to estimate the axial ratios, a/b, and the molecular dimensions of lignin sulfonates at several arbitrarily chosen molecular weights. The corresponding experimental diffusion coefficients, D, were interpolated from the correlating line (Fig. 1), and the diffusion coefficients for spherical particles of the same molecular weights, D_s, were calculated from equation 4. These results (Table V) based on the assumption of a rigid prolate spheroid, suggest that lignin molecules exist in solution as elongated, rod-like particles of approximately constant diameter but of length which increases with increasing molecular weight.

However, since lignin sulfonates are thought by the authors to arise as products of hydrolysis of a more or less infinite network in the wood, it seems improbable that such products would be as ordered in shape as rods of equal diameter. We prefer to regard lignin molecules in solution as non-rigid chains which are penetrated by the solvent as is indicated by homogeneous hydrolysis of lignin sulfonates which proceeds under acidic conditions.[25]

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Lignin sulfonates in solution must be hydrated in view of the undoubted presence of such polar groups as hydroxyl and sulfonate.

Flory and Fox developed the theory[26] of the hydrodynamic behavior of such a chain model, when the chains are linear and are randomly coiled, and indicated that the diffusion coefficient should vary inversely as 0.5 to 0.6 power of the molecular weight. In the present work, a 0.57 power variance is observed (Fig. 1).[27] The sizes of chain model lignin sulfonates of the several molecular weights given in Table V were estimated by using the Einstein equation with diffusion coefficients from the correlating line to obtain the frictional coefficients, $f_0 = kT/D_0$, and from these the root-mean-square end-to-end distances for random linear polymer molecules were calculated using the following equation given by Flory[26]

$$(\overline{r^2})^{1/2} = P^{-1}\frac{f_0}{\eta_0} \qquad (6)$$

TABLE V

ESTIMATED DIMENSIONS OF SODIUM LIGNIN SULFONATES

Mol. wt.	D[a] (cm.²/sec. × 10⁷)	Rigid spheroid model[b] f_s/f	a, Å.	b, Å.	Chain model[c] $(\overline{r^2})^{1/2}$ Å.
10,000	17.0	1.00	28	28	48
20,000	11.5	0.91	75	25	70
40,000	7.7	.70	175	22	105
80,000	5.2	.59	340	24	155
120,000	4.2	.55	425	25	195

[a] Values of D were taken from the correlating line in Fig. 1 at the arbitrarily selected molecular weights. [b] Values of a and b were calculated from the a/b ratio secured from equation 5. [c] The root-mean-square end-to-end distances were obtained by use of equation 6.

The molecular weights reported by Gralen[5] were calculated from observations of sedimentation velocity constants and diffusion coefficients of thioglycolic acid lignin, hypobromite lignin and acetic acid lignin, and in some cases his points fall close to the correlating line in Fig. 1. However, it is difficult to interpret the significance of these results because of their considerable scatter, which perhaps arises from the existence of rather large polydispersity in the samples as indicated by the observed diffuse sedimentation boundary.

For one purified lignin sulfonate preparation, Ritter[7] has reported values for diffusion coefficient and for intrinsic viscosity and these have been used in the following relationship[26] to estimate the molecular weight to be about 22,000 based on the

$$\frac{f_0}{\eta_0} = P\phi^{-1/3}(M[\eta])^{1/3} \qquad (7)$$

random chain model and taking Flory's value of 2.5×10^6 for the constant $\phi^{1/3}P^{-1}$. This value is plotted in Fig. 1 and is in accord with present results.

This apparent fit of the Flory and Fox theory gives some support to the chain model for lignin sulfonates in solution, and may also indicate that chain branching is not extensive.[28] However, since available experimental evidence is insufficient to permit establishment of this model, further studies are being conducted in this Laboratory.

Some properties of fractionated lignin sulfonates have been examined and a few results on the present samples may be reported. Ultraviolet absorption spectra have been characterized as shown in Table I in terms of two empirical absorptivity ratios, A and B, where A is the ratio of the absorptivity at 2800 Å. to that at the wave length of minimum absorption at about 2600 Å., and where B is the ratio of the absorptivity at 2800 Å. to that at 3100 Å. It is found that both of these ratios decrease in an orderly manner with increase in molecular weight of the lignin sulfonates. Weight averages of the absorptivity ratios for the fractions are 1.38 and 3.15 for A and B, respectively, which agree with the values of 1.40 and 3.12 found for the unfractionated material.

Values of the fluorescence depolarization factors, ρ_f, are given in Tables III and IV and it is found that these factors decrease in an orderly manner with increase in molecular weight of the lignin sulfonates. This trend appears to be in agreement with Perrin's theory of polarization of fluorescence[29] and indicates that the fluorescence is an intrinsic property of lignin sulfonates.

Reducing substances in present fractions 1, 3, 5, 7 and 9 were estimated on a salt-free basis to be 2.3, 1.7, 1.5, 1.5 and 1.4% by weight calculated as glucose. Since the initial lignin sulfonate preparation had been dialyzed to remove sugars and other low molecular weight materials substantially to completion, this reducing power probably arises as a result of the presence of carbonyl groups on the lignin sulfonates and corresponds to carbonyl equivalent weights of 7700, 10,400, 12,200, 12,400 and 13,000, respectively. Although these equivalent weights may not be correct on an absolute basis, the data indicate that the frequency of occurrence of carbonyl groups increases as the molecular weight of the lignin sulfonates is decreased.

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