water, and recrystallized from an acetic acid–acetone mixture to obtain 1,3-propylenebis(phenylphosphinic acid), mp 153–155° (lit.[10] mp 157–159°).

Anal. Calcd for $C_{15}H_{18}P_2O_4$ (324.2): C, 55.61; H, 5.61; P, 19.14; neut equiv, 162.1. Found: C, 55.79; H, 5.81; P, 19.91; neut equiv, 166.5.

The yield of diacid was 0.82 g (33%). When the acid is recrystallized from ethanol, an alcoholate which decomposes at about 120° is obtained.

Reaction of PCl_5 with Resin Obtained from 2-Phenyl-1,3,2-dioxaphosphorinane. The resin obtained from the polymerization of 2-phenyl-1,3,2-dioxaphosphorinane with BF_3 etherate (4 g) was mixed with PCl_5 (3.5 g) and the mixture was heated in a sealed tube for 5 hr. The reaction mixture was distilled to obtain $POCl_3$, bp 101°, 1.6 g (48%), γ-chloropropylphenylphosphinic chloride, 1.5 g (30%), bp 150° (0.3 mm) (lit.[3d] bp 125–127° (0.05 mm), and a distillation residue of 2.20 g.

The γ-chloropropylphenylphosphinic chloride was identified by hydrolysis to the corresponding acid, mp 82–84°.

Anal. Calcd for $C_9H_{12}ClPO_2$ (237.0): C, 45.65; H, 4.69; P, 13.10; Cl, 29.51; neut equiv, 218.5. Found: C, 45.17; H, 4.30; P, 13.32; Cl, 29.98; neut equiv, 218.2.

The distillation residue could not be rectified further. It was dissolved in saturated sodium carbonate solution. The resulting solution was filtered from a trace amount of insoluble material and was acidified with dilute HCl. The sticky precipitate which separated was dissolved in acetone and the resulting solution was evaporated to dryness *in vacuo*. The residue as triturated with acetone to obtain 1,3-propylenebis(phenylphosphinic acid), 0.64 g (20%), as a white powder, mp 153–155° (lit.[10] mp 157–159°). The infrared spectrum of this product was identical with that of the diacid recovered from the resin hydrolysate.

Acknowledgments. The authors are grateful to the Esso Research and Engineering Co. for support of this study.

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Lignin. XIV. Gel Chromatography and the Distribution in Molecular Size of Lignin Sulfonates at Several Electrolyte Concentrations[1]

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ABSTRACT: Sodium lignin sulfonates, from gymnosperm woods, were fractionated using Sephadex G-50 gel columns and eluting with distilled water or with 10^{-1} to 10^{-4} *M* NaCl solutions. Molecular weights of certain lignin sulfonate fractions were estimated by ultracentrifuge sedimentation equilibrium and were found to range in an orderly manner from about 70,000 in the mostly excluded initial eluates down to several hundred in the final eluates. For approximately corresponding fractions in the several separations conducted, the elution curves and the estimated molecular weights were similar but not identical and the observed changes occurred sequentially with respect to the molarity of the eluent. The sizes of the lignin sulfonate polymer molecules in terms of equivalent Einstein spheres, r_η, were estimated from the measured molecular weights and from intrinsic viscosities calculated using relationships reported by Goring and coworkers. These ranged from about 7 to 70 Å. For a lignin sulfonate of a particular molecular weight, r_η was estimated to be up to two times greater in water than in 0.1 *M* NaCl solution—behavior expected for an elastic network containing negative charge sites in an environment of varying ionic strength. Support for the concept of change in molecular size with electrolyte concentration is found in the fact that column functioning was correlated satisfactorily in terms of log $[\eta]M_w$ *vs.* V_e, the elution volume; r_η *vs.* K_d, the fraction available of the volume of the gel liquid; and r_η *vs.* the inverse error function complement of K_d.

Lignin is a phenylpropane type of polymer which exists in wood as a three-dimensional network probably chemically bound at some sites to cellulose and/or other carbohydrate materials.[3,4]

By treatment of woody tissue at elevated temperature with an aqueous solution containing bisulfite ions, at least some linkages in the lignin polymer network and lignin–carbohydrate attachments are hydrolyzed. Simultaneously, sulfonate groupings are added to the phenylpropane-type residues to yield soluble lignin sulfonate (LS) molecules which become dissolved in the aqueous solution along with other soluble components of the tissue.[4b]

Several investigators have shown that these soluble LS molecules and other lignin preparations manifest a

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(2) (a) Senior Research Associate, Department of Chemical Engineering, University of Washington, Seattle, Wash. (b) Professor of Chemical Engineering and Dean of Graduate School, University of Washington, Seattle, Wash.

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wide range of polydispersity in molecular weights.[5–8] Recently some low molecular weight LS, or LS-like substances, have been isolated in crystalline form in this laboratory and their chemical structures have been established.[9–11] In the course of these studies some progress was made toward isolation of other lower molecular weight members of the LS polymer series.

Thus the development, during recent years, of the powerful technique of gel chromatography for separation of polymeric materials often primarily on the basis of molecular dimenions has been followed carefully.[12–15]

The interesting initial applications in the Helsinki laboratory of these procedures with Sephadex gels to the separation of LS components have been of particular concern.[16–22]

In view of the achievement and promise inherent in these and other investigations, we have conducted experiments directed primarily toward the concentration or isolation and preliminary characterization of LS-mers ranging up to several thousand in molecular weight. This paper is an introductory report on our studies in this field.

Experimental Section

Sodium Lignin Sulfonates (NaLS). Calcium base spent sulfite liquor, obtained mainly from Western Hemlock (*Tsuga heterophylia*), was steam stripped to remove sulfur dioxide and the fermentable sugars were consumed by alcoholic fermentation. The solution was then decationized with Dowex 50 resin and treated with octadecyldimethylamine to precipitate LS as an insoluble amine salt. This precipitate was separated by centrifugation, resuspended, washed with distilled water, and the NaLS was regenerated by treating the precipitate with sodium hydroxide. The NaLS was partially decationized to pH 3.4, treated with barium hydroxide to precipitate traces of sulfate, centrifuged, adjusted to pH 5 with H^- resin, passed through a column of Na^+ resin (Dowex 50) to replace barium with sodium, spray dried, and then further desiccated over anhydrous calcium sulfate. This procedure and its justification are described in detail elsewhere.[23]

TABLE I
ANALYTICAL CHARACTERISTICS[a] OF SODIUM LIGNIN SULFONATES (NaLS)

Absorptivity, a_{2800}	13.1 l./g cm
Methoxyl, OCH_3	11.4%
Sulfur (total)	6.6%
Sulfur (sulfonate)	5.8%
Sodium, Na	4.2%
Calcium, Ca	0.02%
Nitrogen (Kjeldahl)	0.1%

[a] The analyses are given on the basis of oven dry NaLS.

The NaLS sample for the now-reported study, along with the analytical data shown in Table I, were kindly provided by Mr. V. F. Felicetta of the Puget Sound Division of Georgia Pacific Corp., Bellingham, Wash. The observed small percentage of nitrogen may be attributable to residual octadecyldimethylamine, or its derivative.

Fractionation of Sodium Lignin Sulfonates. Sephadex G-50 (200 g of particle size 20–80 μ; lot no. to 4759) was soaked overnight in 0.1 *M* NaCl solution. A Pyrex glass column (33.8 mm i.d.) was packed with the swollen gel. The height of the gel in the column was about 210 cm and the bed volume was about 1890 ml. Packing, stabilization, and sample application were conducted as described elsewhere.[24]

Fractionations, using 100 mg of NaLS dissolved in 5 ml of eluent, were carried out. In the several experiments reported, eluents consisting of 0.1, 0.01, 0.001, and 0.0001 *M* NaCl solution and distilled water were used. In each case, series A, prior to the application of the NaLS sample, the Sephadex gel column was equilibrated with the NaCl solution until the eluate approached the conductivity of the eluent. Of the eluate, some 150–200 fractions of 10 ml each were collected in an automatic fraction collector.

In another series of experiments, series B, after equilibrating the gel column with 0.1, 0.01, 0.001, or 0.0001 *M* NaCl solution, and then adding the NaLS sample, the elutions were conducted with distilled water. Conductivities of the eluates were also measured.

Characteristics of the columns used were approximately the following (symbols are defined in the Glossary given later): V_t = 1890 ml; V_g = 125 ml; V_0 = 510 ml, and V_i = 1250 ml. Liquid flowed through the column at rate of approximately 0.06–0.10 ml/cm² min. The temperature was 24 ± 1°.

The eluate was continuously analyzed to determine absorbance at 2800 Å using a LKB 8300A Uvicord II ultraviolet absorptiometer (manufactured by LKB-Produkter AB, Stockholm, Sweden). In some cases the analyses were conducted manually by use of a Beckman DU spectrophotometer. Conductivity measurements were made manually on some eluates and, in some cases, continuously using a recording LKB 8300B conductolyzer.

Molecular Weight Estimation. Weight average molecular weights, M_w, were determined by equilibrium sedimentation in Spinco Model E ultracentrifuge using the short-column technique of Van Holde and Baldwin.[25] Column heights of solutions were about 2.5–3 mm and the volume of sample solution was about 0.1 ml. The runs were made at close

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TABLE II
CUMULATIVE WEIGHTS OF NaLS ELUTED AT V_e BY AQUEOUS SODIUM CHLORIDE SOLUTIONS

V_e, ml	% eluted at 0.1 *M*	0.01 *M*	0.001 *M*	0.0001 *M*	H_2O
		Series A[a]			
500	8	1	29	22	
750	48	61	64	73	
1000	61	73	74	92	
1250	79	81	80	94	
1500	85	87	84	97	
1750	89	93	88	100	
2000	90	96	91	...	
2250	...	99	...	...	
2500	...	100	...	...	
		Series B[a]			
500	...	3	2	19	1
750	48	68	70	73	67
1000	62	79	81	88	85
1250	73	85	86	96	92
1500	82	90	91	99	96
1750	91	100	98	...	...
2000	101	...	...	...	...
2250	...	...	...	...	...
2500	...	...	...	...	...

[a] In all cases, the columns were equilibrated with NaCl solutions of the molarity shown. For series A, elutions were conducted with the NaCl solutions of the same molarity as the equilibrations. For series B, elutions were conducted always with distilled water.

to 20°. The NaLS solution examined was adjusted to 0.1 *M* NaCl.

A split beam scanning absorption system[26] was used to follow the redistribution of solute molecules in the centrifuge cell. Scans were made using monochromatic light at 2800 Å with 2 mm slit width. Initial absorbance of solutions was about 0.4 which corresponded to a concentration of 30 mg of NaLS/l. Actual pen deflection measurements (mm) were translated into concentration (absorbance units).

Up to 15 samples were analyzed in a single experiment through the use of multichannel cells described by Yphantis.[27] Suitable volumes of fluorinated hydrocarbon FC-43 were used to stagger the images of the cells. Absorption counterbalance provided references in the cell scan for the determination of the magnification factor of the optical system and also of the distances from the axis of rotation. After attaining equilibrium at a selected speed, the cell images were scanned.

Molecular weights were calculated from the slope of the curve using the expression

$$M_w = \frac{2RT}{(1 - \bar{v}\rho)\omega^2} \frac{\mathrm{d} \ln C}{\mathrm{d}(x^2)} \qquad (1)$$

A Fortran IV program for the IBM 7094 written by Mr. Roger Wade of the Biochemistry Department of the University of Washington was adapted for the computations. The partial specific volume of NaLS was taken to be 0.610 ml/g.[6] A straight line fit to the plot of ln C *vs.* x^2 gave the slope d ln $C/\mathrm{d}(x^2)$ in the above equation. The values of C were multiplied by a factor of 10 in order to prevent negative logarithms in computer calculations. Standard deviations in percentages were recorded.

Glossary

a	Absorptivity of NaLS, 1/g cm
C	Concentration of NaLS, recorder pen deflections converted into absorbance units
K_d	Fraction of the imbibed solvent available to the macromolecules of a given size
M	Molarity
M_w	Weight average molecular weight
N	Avogadro constant, 6.0225 × 10^{23} mol^{-1}
r_η	Radius of the equivalent Einstein sphere, Å
R	Gas constant, 8.3143 × 10^7 erg/°K mol
T	Temperature, °K
$\bar{v}$	Partial specific volume, ml/g
V_e	Elution volume of the solute, ml
V_g	Volume of the gel matrix, ml
V_i	Volume of the liquid imbibed in gel matrix, ml
V_0	Volume of the interstitial liquid, ml
V_t	Total volume of the gel bed in column, ml
x	Distance from the axis of rotation, cm
$[\eta]$	Intrinsic viscosity, dl/g
ρ	Density of solution, g/ml
ω	Angular velocity of rotor, rad/sec

Results and Discussion

Preliminary experiments conducted to fractionate a sample of NaLS using a column of Sephadex G-50 yielded, under certain conditions, elution curves manifesting peaks in NaLS concentrations which suggested partial separations of individual mers, or of groups of mers, of similar characteristics. However, satisfactory replication of results was not obtained in certain trials conducted with a similar amount of NaLS sample but not identical concentration of electrolyte in the eluent.

Further experimentation, which will be described below, demonstrated that adequate reproducibility in fractionation results was obtained when the molarity or electrolyte concentration in the eluent was maintained approximately at a fixed level and, moreover, particularly promising separations of LS-mers, or groups of mers, arose when the electrolyte concentration was maintained at a certain apparently optimum level.

The several fractionations were conducted using as eluents 0.1, 0.01, 0.001, and 0.0001 *M* sodium chloride solutions, as well as distilled water itself. Tables II, III, and IV summarize the results secured.

The type of elution curve obtained, in one of the series A experiments, is shown in Figure 1A. First, at about V_e = 490 ml, a large LS peak emerges. As indicated by the molecular weight evidence given below, this LS consists primarily of molecules so large that they are excluded almost completely from the solvent within the gel; thus for this column, V_0 = 490 ml. As V_e increases, the concentration of LS declines and a number of small peaks become evident. However, it is observed that a significant amount of LS is still appearing in the eluate after V_e reaches and exceeds V_i, and this result indicates that the phenomenon of adsorption of LS does exert some influence upon the progress of the elutions. The peak in conductivity at about V_e = 1580 ml may arise because of the presence of some sugar acids and this material is being further investigated.

In series B, distilled water was used as the eluent after equilibrating the column with a particular electrolyte solution and then adding the NaLS sample. Here, as Figure 1B shows, approximately "plug" flow of the water occurs to yield a substantial LS peak at V_e where

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TABLE III
CUMULATIVE WEIGHTS AND SOME CHARACTERISTICS OF NaLS ELUTED AT V_e BY AQUEOUS SODIUM CHLORIDE SOLUTIONS (SERIES A)[a]

Expt	V_e, ml	Cumulative NaLS eluted, %	M_w[c]	SD, %	$[\eta]_M$, dl/g	r_η, Å	K_d
A, 0.1[b]	530	8	62,096	6.9	0.055	38	0
	610	36	15,534	0.6	0.035	21	0.06
	910	56	6,740	2.0	0.027	14	0.31
	1110	66	4,545	1.8	0.024	12	0.47
	1310	73	3,065	0.6	0.021	10	0.63
	1410	76	2,190	2.1	0.019	9	0.71
	1620	82	2,032; 2,012	1.1; 1.4	0.018	8	0.88
A, 0.01	520	16	40,232	4.3	0.090	39	0
	570	45	11,084	0.4	0.060	22	0.02
	870	67	2,937	5.1	0.039	12	0.28
	1070	76	2,422	1.2	0.037	11	0.44
	1420	86	1,374; 1,223	1.3; 4.3	0.031	9	0.73
A, 0.001	480	15	59,275	4.9	0.178	55	0
	550	49	8,153	0.7	0.094	23	0.06
	840	68	1,610	0.6	0.056	11	0.29
	1280	81	1,384; 1,112	1.5; 1.8	0.053	10	0.65
	1940	90	797; 526	1.8; 0.8	0.045	8	1.18
A, 0.0001	490	15	37,176	6.9	0.232	52	0
	640	59	6,503	0.7	0.133	24	0.12
	790	76	2,486; 2,632	1.1; 0.6	0.099	16	0.24
	990	88	1,552	0.5	0.084	13	0.40
	1100	92	648; 828	0.6; 2.3	0.069	10	0.49
	1490	96	730; 785	0.8; 2.9	0.066	9	0.81
	1540	99	408	1.7	0.055	7	0.85

[a] In all cases, the columns were equilibrated with NaCl solutions of the molarity shown. For series A, elutions were conducted with the same NaCl solutions as the equilibrations. [b] Molarity of NaCl in the eluent. [c] The molecular weights are presented as printed out by IBM digital computer. The first two figures are significant.

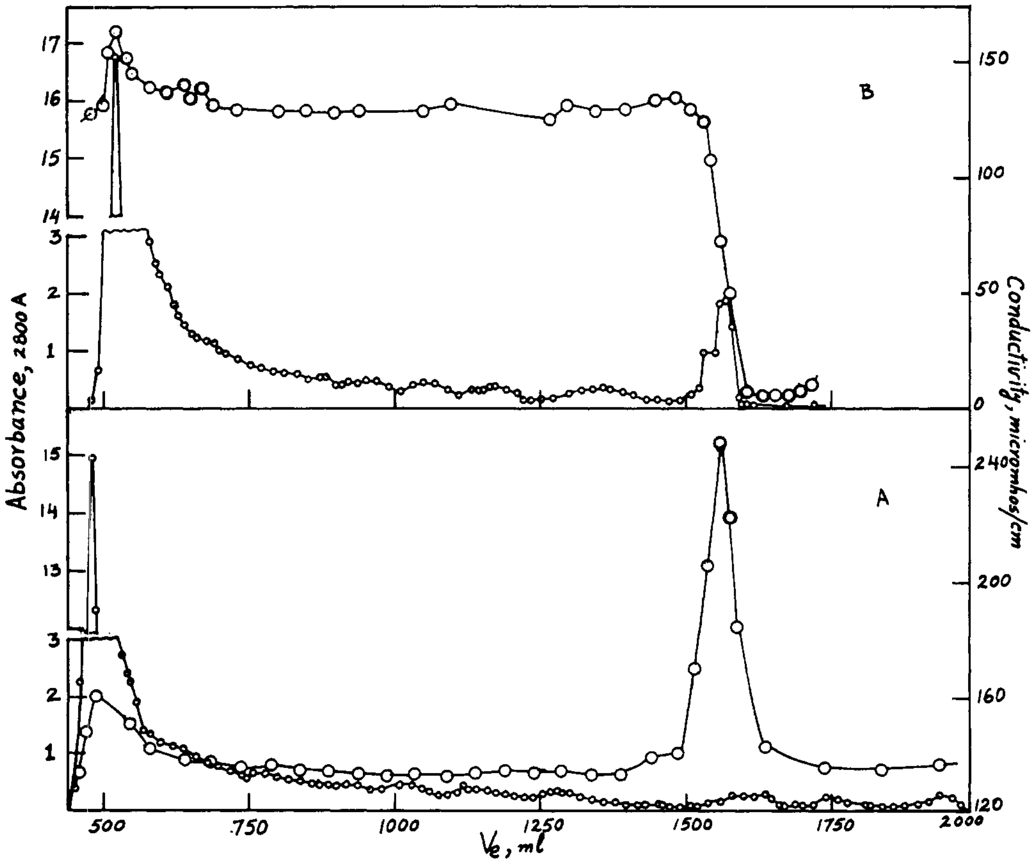

Figure 1. Elution curves for sodium lignin sulfonates. For A, the column equilibration and elution were conducted with 0.001 M NaCl solution. For B, the column equilibration was conducted with 0.001 M NaCl and the elution with distilled water: ○, V_e vs. absorbance; ○, V_e vs. conductivity.

TABLE IV
CUMULATIVE WEIGHTS AND SOME CHARACTERISTICS OF NaLS ELUTED AT V_e BY AQUEOUS SODIUM CHLORIDE SOLUTIONS (SERIES B)[a]

Expt	V_e, ml	Cumulative NaLS eluted, %	M_w[c]	SD, %	$[\eta]_M$, dl/g	r_η, Å	K_d
B, 0.1[b]	530	15	58,656	5.5	0.054	37	0
	590	35	12,774	1.9	0.033	19	0.05
	1090	66	3,678	1.2	0.022	11	0.45
	1500	82	2,728	4.3	0.020	10	0.78
	1780	93	1,338; 1,325	0.9; 0.7	0.016	7	1.01
B, 0.01	520	13	48,551	7.9	0.096	42	0
	630	58	7,695	0.9	0.053	19	0.09
	880	74	2,871	1.0	0.039	12	0.29
	1180	82	2,249; 2,127	2.3; 0.6	0.036	11	0.53
	1620	95	1,143; 1,036	1.1; 0.6	0.029	8	0.89
B, 0.001	520	20	31,398	10.4	0.145	42	0
	670	63	4,960	1.5	0.080	19	0.12
	880	77	2,417; 2,282	0.5; 0.7	0.064	13	0.29
	1050	83	1,776; 1,326	0.4; 3.8	0.058	12	0.43
	1350	89	1,053; 896	0.9; 2.2	0.049	9	0.67
	1570	96	707; 603	2.8; 2.0	0.043	8	0.85
B, 0.0001	500	19	73,196	4.5	0.288	69	0
	590	52	9,861	1.1	0.152	29	0.07
	840	80	2,433; 2,210	0.4; 0.8	0.097	16	0.27
	980	87	1,521; 1,190; 998	2.5; 2.6; 4.4	0.083	13	0.39
	1110	92	1,013	0.9	0.073	11	0.49
	1250	96	674; 405	4.6; 1.0	0.064	9	0.60
	1420	99	...	...	...	..	0.74
B, H_2O	530	14	59,359	4.1	0.323	67	0
	610	46	9,646	0.9	0.181	30	0.06
	970	85	1,027; 1,170	2.6; 2.1	0.088	11	0.35
	1090	89	929; 1,017	3.8; 2.1	0.085	11	0.45
	1230	92	776; 580; 669	1.4; 1.2; 2.5	0.081	10	0.56

[a] In all cases, the columns were equilibrated with NaCl solutions of the molarity shown. For series B, elutions were conducted always with distilled water. [b] Molarity of NaCl in the eluent used for equilibration. [c] The molecular weights are presented as printed out by IBM digital computer. The first two figures are significant.

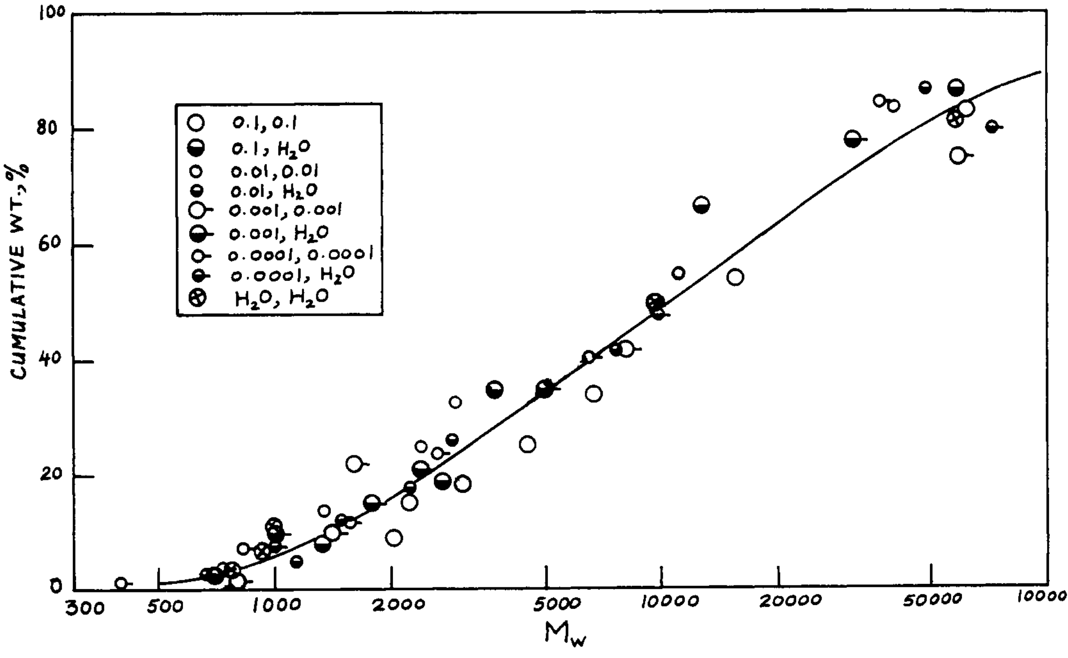

Figure 2. Distribution of molecular weights of initial sodium lignin sulfonate sample (from the data of columns 3 and 4 in Tables III and IV). The legend shows the molarities of NaCl solution used for equilibrations and elutions, respectively.

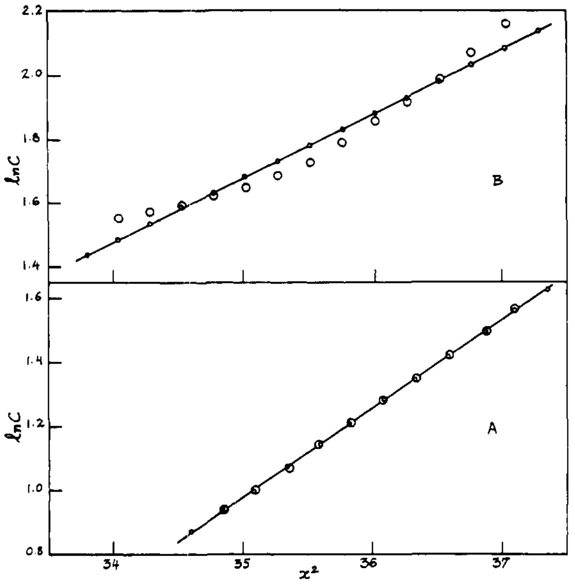

Figure 3. Plot of ln C *vs.* x^2 for molecular weight determination by sedimentation equilibrium method: O, experimental data; o, straight line fit to observed data. In A, $M_w \simeq 3000$; SD = 0.6%. In B, $M_w \simeq 62{,}000$; SD = 6.9%.

the conductivity measurements indicate a water "breakthrough"; thereafter, the LS concentration rapidly approaches zero. Thus sharp "breakthrough" takes place at successively decreasing values of V_e = 1750, 1610, 1510, or 1420 ml as the molarity of NaCl solution used for equilibration is reduced from 10^{-1} to 10^{-4} M and to distilled water in a stepwise manner. The data in columns 2, 3, 4, 5, and 6 of series B in Table II illustrate this phenomenon clearly.

To facilitate comparison, elution data for each experiment were plotted and graphically integrated. Cumulative NaLS weights were estimated using the absorptivity value as a_{2800} = 13.1 l/g cm, which is in approximate agreement with other reported values.[3,4a,6] Integrations were conducted up to values of V_e corresponding to several arbitrarily chosen limits, and to the center of "peaks" when evident. Results are given in Tables II, III, and IV. Over-all recovery amounted to about 90–101% of the weights of NaLS material added and, in most cases, low recovery was associated with too-soon cessation of eluate collection.

The molecular weights of the NaLS collected at most of the peaks, and at certain other V_e values, were estimated by the short column sedimentation equilibrium technique of Van Holde and Baldwin[25] since this seemed to be the best available way to treat the polymer electrolyte systems in this molecular weight range. Distribution of NaLS concentration at sedimentation equilibrium was estimated by absorption optics at 2800 Å and the procedure functioned successfully with NaLS at such low dilutions that concentration effects could be neglected. Centrifugal field strengths were suitably selected and the runs were of sufficiently long duration to attain equilibrium. Insufficient data were collected to permit extrapolation of molecular weights to zero centrifugal fields but this field effect, if real, seemed not large.

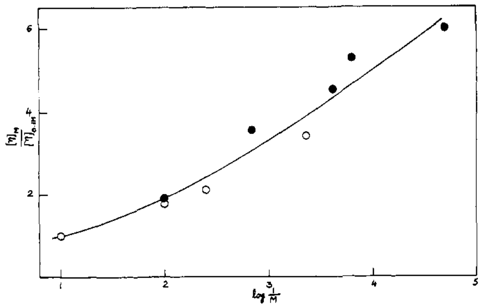

Figure 4. Relationship between [η] and the molarity of the electrolyte. Two sets of data from ref 29 are averaged. This curve was used to estimate increase in [η] with decreasing molarity of electrolyte, NaCl solution.

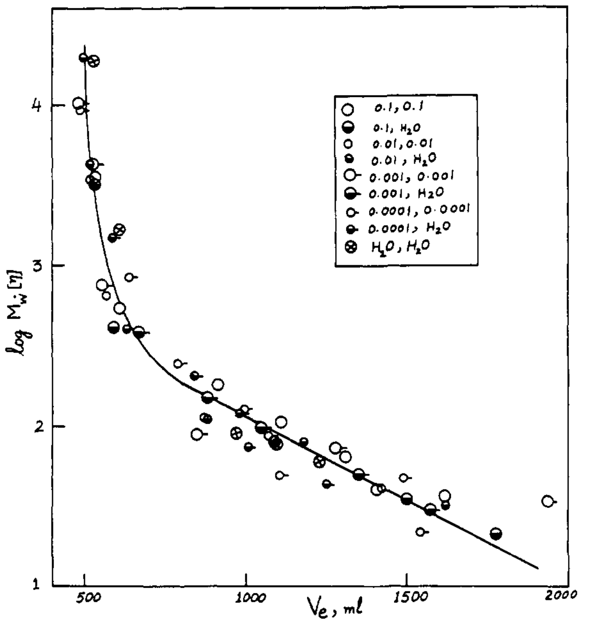

Figure 5. Plot of log [η]M_w *vs.* the elution volume, V_e (from the data of columns 2, 3, 4, and 6 in Tables III and IV). The legend shows the molarities of NaCl solution used for equilibrations and elutions, respectively.

The cumulative weight distributions found for the molecular weights of the NaLS in all nine fractionations are shown in Figure 2. Here it is evident that the several points fall close to the single curve which has been drawn to represent the molecular weight distribution existing in the initial unfractionated NaLS sample. The now-reported distribution is in approximate accord with the results of earlier work conducted in this laboratory and elsewhere,[7,8,28] although more detailed information is now given concerning the molecular weight range of 500–10,000.

The degree of polydispersity in molecular weight within a particular NaLS fraction was also examined. This evaluation was made in terms of the percentage standard deviation, per cent SD, in NaLS molecular weight calculated from the departure of the "best fit" straight line from the experimental points. Figure 3A shows a ln C *vs.* x^2 graph. Only small polydispersity, *i.e.*, 0.6% SD, is shown for the NaLS ($M_w \simeq 3000$)

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(28) J. Moacanin, H. Nelson, E. Back, V. F. Felicetta, and J. L. McCarthy, *J. Amer. Chem. Soc.*, **81**, 2054 (1959).

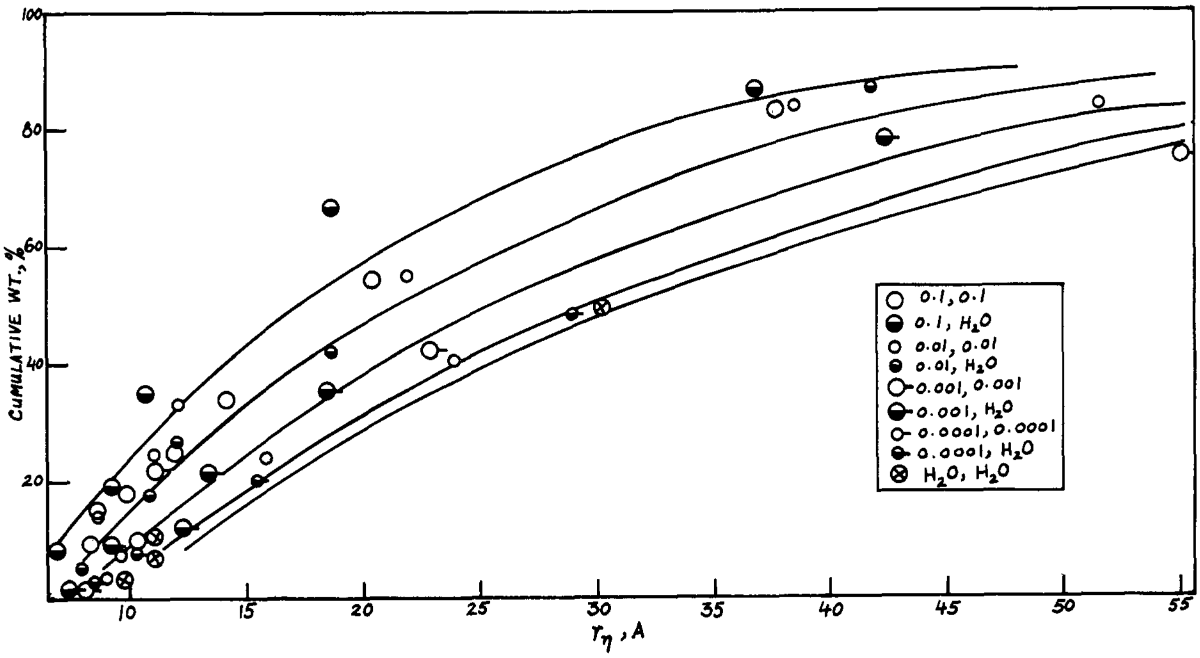

Figure 6. Relationship between the molecular radii, r_η, and the cumulative weights of NaLS (from the data of columns 3 and 7 in Tables III and IV). The curves were drawn using smoothed M_w data of Figure 2 and computing r_η in 0.1, 0.01, 0.001, and 0.0001 M NaCl and distilled water employing eq 2, Figure 4, and eq 3, respectively. The legend shows the molarities of NaCl solutions used for equilibrations and elutions, respectively.

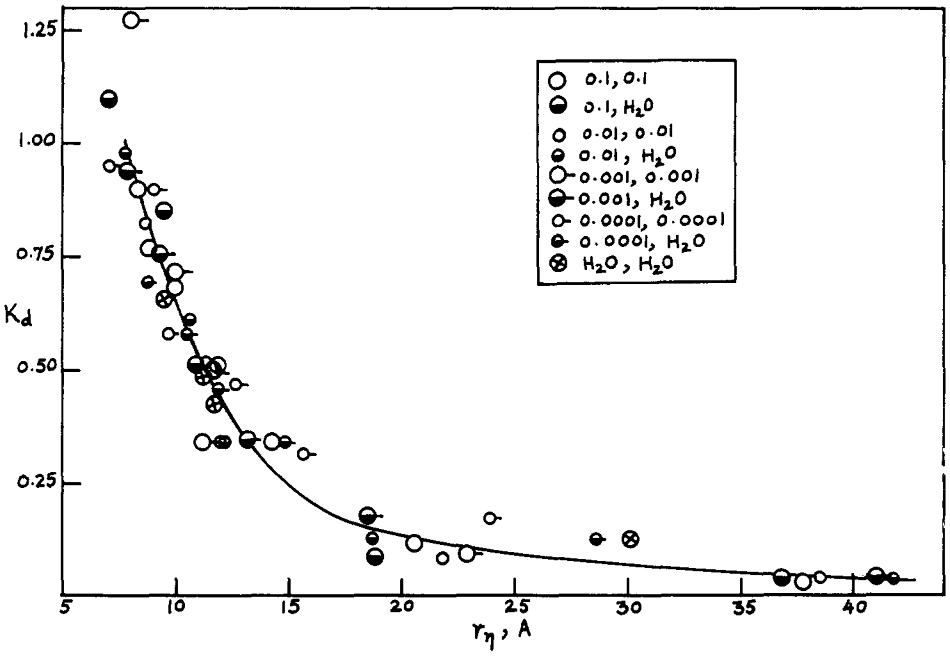

Figure 7. Plot of r_η vs. K_d (from the data of columns 7 and 8 in Tables III and IV). The legend shows the molarities of NaC solutions use for equilibrations and elutions, respectively.

which was collected at $V_e = 1310$ ml and should be well fractionated. However large polydispersity, *i.e.*, 6.9% SD, is evident for the NaLS ($M_w \simeq 62{,}000$) which was collected at $V_e = 530$ ml where almost total exclusion occurred and thus the sample was not effectively fractionated.

In view of the hydrodynamic importance of the intrinsic viscosity of the NaLS solutions, its determination was considered. However, it soon became recognized that usefully precise measurements would be difficult to obtain by direct experimentation because only a small change in viscosity is to be expected in view of the low molecular weight and apparently small asymmetry of the NaLS molecules. In an alternative procedure, the intrinsic viscosities, $[\eta]$, were calculated using eq 2.[8] We have assumed that this equation is

$$[\eta]_{0.1M} = 0.0016 M_w^{0.32} \qquad (2)$$

applicable to the low molecular weight NaLS molecules studied in this paper although the experimental data of Goring, *et al.*,[8] extended only down to the range of about 50,000.

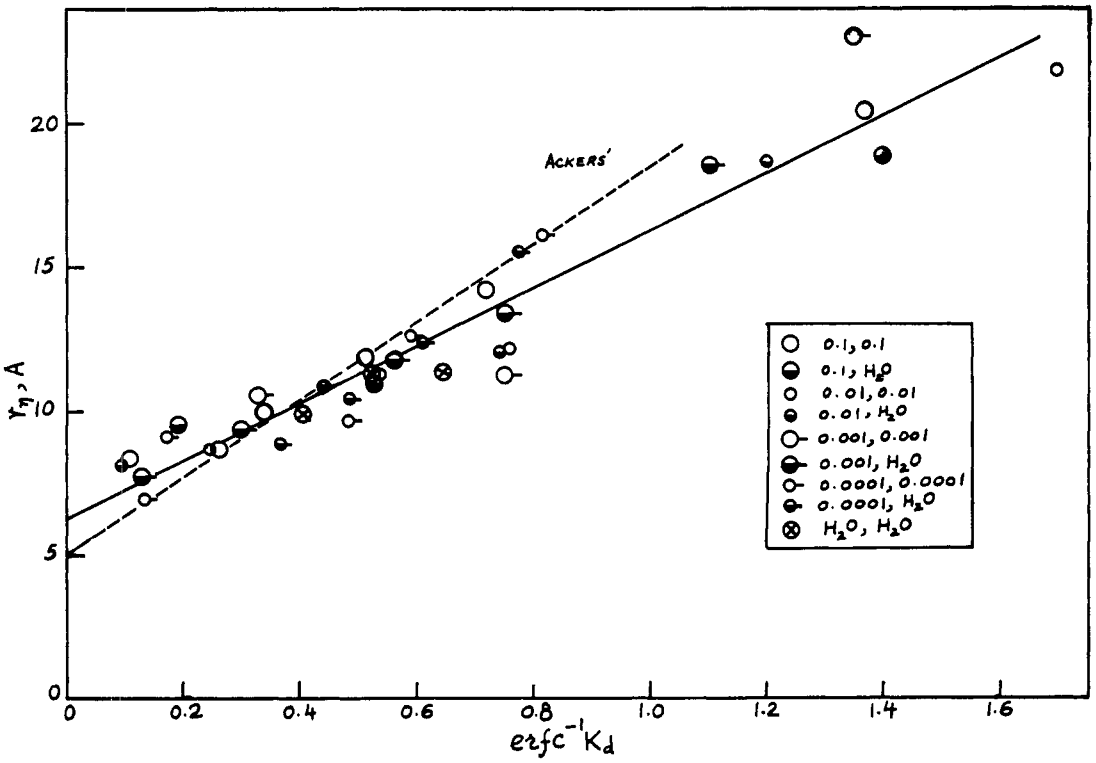

Figure 8. Graph showing linear relationship between r_η and erfc^{-1} (K_d). The values of the inverse error function complement of K_d were read from the "Tables of the Error Function and Its Derivatives."[34] The legend shows the molarities of NaCl solution used for equilibrations and elutions, respectively.

For the present purposes, the $[\eta]$ values at electrolyte concentrations, other than 0.1 M NaCl, were needed and these were taken from a graph (Figure 4) of normalized intrinsic viscosity against the logarithm of reciprocal of molarity of NaCl from the data of Rezanowich and Goring.[29]

Several relationships have been proposed relating an elution volume parameter with size and/or weight of the macromolecules. These are summarized by Pecsok and Saunders.[14] Most of these relations hold for a limited range of molecular weights. Recently, Grubisic, Rempp, and Benoit[30] suggested that a plot of the logarithm of the product of $[\eta]M_w$ *vs.* V_e should yield a linear relationship. A similar plot of elution data on NaLS is shown in Figure 5. The initial part of the curve with high slope represents the material which is excluded from the column and is not effectively fractionated. However, a linear relationship holds when the NaLS are fractionated successfully as represented by the latter part of the curve with smaller slope.

The molecular sizes of the LS molecules separated in certain fractions were also estimated from the available molecular weight and intrinsic viscosity data using eq

$$r_\eta = \left[\frac{30M_w[\eta]}{\pi N}\right]^{1/3} \tag{3}$$

3.[29] The results (Figure 6) indicate that the effective radius of a NaLS molecule may almost double as the electrolyte environment is changed from 0.1 M NaCl to pure water. This appears to be due to the expansion or swelling of the NaLS macromolecules.[29,31] Gupta and Goring[32] have shown that water soluble alkali lignin is also a swellable polyelectrolyte.

The characteristics of the Sephadex G-50 gel itself may be represented in terms of the data now available. Thus, for a particular LS fraction, K_d was calculated from the observed V_e and knowledge of column characteristics, and r_η was calculated from the measured molecular weight and the intrinsic viscosity (eq 3) described above. The results (Figure 7) indicate, although no doubt only crudely, the distribution of accessible solvent volumes in Sephadex G-50 in terms of the effective radii of the pores in the gel. Some 80% of the solvent in the gel seems to be accessible to solutes with an effective radius of about 7 Å, whereas only 20% is accessible to solutes of about 16 Å. Thus it is for molecules with effective radii between these two sizes that the Sephadex G-50 functions particularly well in fractionation.

An alternative and elegant way to characterize the G-50 gel is by use of the procedure recently described by Ackers.[33] Accordingly, the fraction K_d of the internal volume V_i that is penetrable by a solute molecule of radius r_η can be represented by a Gaussian probability curve and related to the error function complement (erfc) of the Gaussian distribution. Thus

$$K_d = \left[\frac{r_\eta - r_{\eta 0}}{s}\right] = 1 - \frac{2}{\sqrt{\pi}}\int_0^{(r_\eta - r_{\eta 0})/s} e^{-r_\eta^2} dr_\eta \tag{4}$$

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(30) Z. Grubisic, P. Rempp, and H. Benoit, *Polym. Lett.*, **5**, 753 (1967).

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(33) G. K. Ackers, *J. Biol. Chem.*, **242**, 3237 (1967).

Table V

Constants in eq 5	r_{η_0}	s
Present work	6	10
Ackers' work[a]	5	13

[a] See ref 33.

$$r_\eta = r_{\eta_0} + s\ \mathrm{erfc}^{-1}(K_\mathrm{d}) \qquad (5)$$

The constant r_{η_0} is the position of the maximum ordinate of the distribution, s the measure of standard deviation, and erfc^{-1} the inverse error function complement.

Ackers showed for several gels that a plot of r_η *vs.* $\mathrm{erfc}^{-1}(K_\mathrm{d})$ was linear and that r_{η_0} and s could be taken to be calibration constants for a given gel. In present work, the available values of $K_\mathrm{d} = (V_\mathrm{e} - V_\mathrm{o})/V_\mathrm{i}$ were graphed in terms of the inverse error function complement, $\mathrm{erfc}^{-1}(K_\mathrm{d})$,[34] *vs.* the corresponding estimated molecular radii, r_η, to yield the approximately linear relationship shown in Figure 8. Constants found for this line, together with those estimated from Figure 2 of Ackers' paper[33] are given in Table V. The proximity of r_{η_0} and s values found in the present work with those reported by Ackers—although his plot is based on data from fractionation of dextrans—gives support to the above discussed concepts of the molecular size and the swelling relationships of NaLS molecules. Our experimental results seem to provide further evidence favoring Goring's interpretations as well as the present ideas concerning the mechanism of gel chromatography separations.

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Properties of Ethylene–Acrylic Acid Copolymers

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ABSTRACT: The effects of carboxylic acid comonomers on polyethylene are studied using calorimetry, dynamic mechanical testing, and infrared spectroscopy. Infrared studies indicate that the fraction of acid groups hydrogen bonded is determined by a temperature-dependent equilibrium with thermodynamic parameters similar to small molecule acids in ordinary solvents. During elongation, the planes of the acid dimer become perpendicular to the direction of stretch. The melting points of the copolymers are tested using Flory's equation. The results show that the acid groups are not crystallizable, and the dimerization takes place in the amorphous phase. The increases in T_g with increasing acid content as determined by E'' are more correctly described by cross-linking equations than by the simple copolymer relationships.

A variety of effects arise from the incorporation of comonomers with functional pendent groups in otherwise nonpolar vinyl polymers. In the cases of carboxylic acids, alcohols, amines, or amides, changes in physical properties are often identified with the hydrogen bonded structures formed. Systematic investigation of how relatively isolated functional groups alter polymer behavior is necessary in understanding the behavior of systems where they are major constituents.

Early investigations have shown that the presence of carboxylic acid or carboxylate comonomers generally increased matrix cohesion but did not lead to mechanically stable networks.[1-3] The picture of acid dimerization and acid–base interactions in amorphous polymers has more recently been refined. Below T_g the functional groups are highly associated and immobile. Above T_g chain mobility allows relatively free diffusion of the groups and the fraction associated is determined by a temperature-dependent equilibrium.[4-6] Superficially this latter behavior is identical with that of small molecule analogs in nonpolar solvents. The thermodynamic parameters of the association, however, may differ from the low molecular weight systems.

In this work the effects of acrylic acid comonomer on the properties of branched polyethylene are discussed where the additional variable of crystallinity is present. Several other authors have discussed such changes for the case of methacrylic acid and its metal salts. These experiments have shown that ionization of the acid depresses crystal formation more severely than does the

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(6) E. P. Otocka and F. R. Eirich, *ibid.*, in press.