solvents. Their density and nondraining nature minimize size variability with changes in solvent quality.

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Lignin. 19. Kraft Lignin Component Conformation and Associated Complex Configuration in Aqueous Alkaline Solution†

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ABSTRACT: Discrete kraft lignin components behave ideally when eluted with 0.10 M aqueous NaOH from a cross-linked dextran gel: fractions with apparent polydispersity indices of 1.04 ± 0.01 are described by elution profiles that are close to a Gaussian shape. Of those selected for the present study, the weight-average molecular weights ($\bar{M}_w$) in aqueous solution at pH 9.5, ionic strength 0.10 M, were found by ultracentrifuge sedimentation equilibrium to vary between 1680 and ~140 000. On the basis of a logarithmic-normal component distribution, the polydispersities of the fractions during chromatographic elution have been compared with those observed under the conditions imposed for the absolute molecular weight determinations. Below the excluded limit for the gel, the plot of log $\bar{M}_w$ vs. column retention volume is linear and parallel to that for poly(styrenesulfonates). The kraft lignin components adopt an appreciably expanded random coil conformation in aqueous alkaline solution; there is no indication of a hydrodynamic effect arising from long-chain branching. The hydrodynamic behavior, on the other hand, of associated kraft lignin complexes is in accord with a flexible lamellar configuration, which is distinct from the conformation of the discrete components.

Introduction

It has been established that one of the most prominent physicochemical properties of lignin components is a pronounced tendency to form high molecular weight associated complexes.[1-4] Such effects dominate the colligative behavior of lignin samples under a variety of conditions ranging from nonaqueous[3] to aqueous alkaline solutions.[4] Furthermore, association, a reversible phenomenon between lignin components, can be complicated by nonreversible aggregation between the resulting complexes.[5-7]

Among lignin derivatives, the most abundant are the industrial byproducts formed under the relatively severe degradative conditions encountered during the kraft pulping process (typically at 170 °C for 5 h in aqueous solution containing 45 g L^{-1} NaOH and 12 g L^{-1} Na_2S). These kraft lignins are thought to have undergone significant structural modifications compared with the native polymer, a view based largely upon appropriate model compound studies[8] that have been partly confirmed by the structures of monomeric and dimeric components identified as being present in the spent liquor.[9]

Size-exclusion chromatography of kraft lignin samples using cross-linked dextran (Sephadex G series) gels with 0.10 M aqueous NaOH generates profiles that represent molecular weight distributions generally within an order of magnitude of those for the discrete components.[3,4] Appreciable variations may be expected in the effective volumes occupied by associated complexes and individual components of the same molecular weight. These may affect the accuracy with which a single calibration curve can be used to characterize the elution profiles of lignin samples differing only in their degree of association. The present work was directed toward investigating the hy-

† All experimental work was conducted at the University of Washington. The analysis of the results was developed by S.S. after transferring to his present position at the University of Minnesota, where the manuscript was also mainly written.

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drodynamic characteristics of kraft lignin species which are reflected in their behavior under such chromatographic conditions.

Experimental Section

Kraft Lignin. The gymnosperm kraft lignin was isolated from kraft black liquor donated by the Weyerhaeuser Co. from the mill at Longview, WA, which utilizes mainly Douglas fir (*Pseudotsuga taxifolia*) wood. Diluted kraft black liquor (1800-mL portions containing 3 g L^{-1} dissolved solids after filtration through a VW&R crepe white paper to remove residual fibrous materials) was acidified with 1.0 M aqueous H_2SO_4 (0.5 mL min^{-1}) to pH 2.5. The resulting solution (containing suspended solids) was centrifuged, and the precipitate was washed thoroughly by resuspending in aqueous solution at pH 2.5 and centrifuging. The sample thus obtained was redissolved in the minimum volume of aqueous solution at pH 8.5 and freeze-dried.

Organosolv Lignin. The Organosolv lignin from red alder (*Alnus rubra*), used for comparative purposes in these studies, was donated by Professor K. V. Sarkanen and co-workers, College of Forest Resources, University of Washington. It was prepared[4] by subjecting wood chips to the hydrolytic conditions encountered in 75% aqueous dioxane (10:1 (v/w) liquor–substrate) at 140 °C for 2 h in the presence of 6×10^{-3} M $AlCl_3$. After the pulping reaction was stopped, removal of the organic cosolvent resulted in the precipitation of the partially degraded red alder Organosolv lignin components from solution into a form that could be conveniently filtered.

Poly(styrenesulfonates). The poly(styrenesulfonate) standards were obtained from Pressure Chemical Co. and used without further purification or fractionation. Except for the 1600 molecular weight standard with an index of 1.25, their polydispersity indices were given as 1.10.

Preparative Size-Exclusion Chromatography. Preparative fractionation of the kraft lignin (10 g) according to molecular size was accomplished by eluting with carbonate-free 0.10 M aqueous NaOH from Sephadex G100 (Pharmacia). Two 70 × 5.0 cm (cylindrical) columns connected in series were found to provide a capacity sufficient to accommodate 1.0 g of the sample containing 3.5% (w/w) *p*-nitrophenol as a standard marker. Gel batches were allowed to swell at room temperature for 3 days in eluant before packing in order to ensure long operational life for the column system. A single-beam LKB 8300A Uvicord II photometer (LKB-Produckter AB, Stockholm), set to monitor at 280 nm as a detector at the column outlet, maintained base line stability within acceptable tolerance limits.

For each fraction, the apparent relative retention volume, V_R (viz., $(V - V_0)/(V_{pNP} - V_0)$, where V and V_{pNP} are the retention volumes of the fraction and *p*-nitrophenol and V_0 is the void volume), was determined with respect to the marker from direct cumulative volume measurements. After having been incubated as 0.10 M aqueous NaOH solutions containing roughly 0.5 g L^{-1} kraft lignin components for ~1500 h at 5 °C, the individual fractions from all ten preparative chromatographic runs were matched according to their apparent V_R values and combined. These combined kraft lignin fractions were passed through a BioRad AG 50W-X8 cation exchange resin (BioRad Laboratories) in its hydrogen form and freeze-dried following careful basification of the eluant solutions to pH 7.0.

Analytical Size-Exclusion Chromatography. A 70 × 2.5 cm (cylindrical) column was found to be adequate for analysis of both the lignin fractions and samples. An ISCO UA-5 monitor with a Type 6 double-beam optical unit was set to operate at 280 nm as a detector at the column outlet.

Among the combined kraft lignin fractions obtained by preparative chromatographic work, 11 were individually eluted with carbonate-free 0.10 M aqueous NaOH from Sephadex G100. The profiles exhibited sharp rises to their respective maxima, while the extents of tailing became progressively more marked with increasing molecular weight of the combined fraction. A paucidisperse fraction (in 0.10 M aqueous NaOH solution) was secured from a narrow region about each peak maximum for molecular weight determination by ultracentrifuge sedimentation equilibrium analysis.

The behavior of these paucidisperse kraft lignin fractions in the analytical size-exclusion chromatographic system was investigated by eluting portions of the respective stock solutions from Sephadex G100 with 0.10 M aqueous NaOH in the presence of appropriate low and high molecular weight markers. Thus the fractions characterized by weight-average molecular weights below 30 000 were coeluted with *p*-nitrophenol and a blue dextran (Pharmacia) of molecular weight sufficiently high to be entirely excluded from the gel. On the other hand, in order to avoid interference from an overlapping blue dextran peak, the fractions with weight-average molecular weights above 60 000 were eluted in the presence of the low molecular weight marker alone since their profiles appeared in a region close to the excluded limit.

The behavior of the poly(styrenesulfonates) in the Sephadex G100/0.10 M aqueous NaOH system was investigated in the same manner. The elution characteristics of guaiacylglycerol β-(2-methoxyphenyl) ether, diisoeugenol, and *p*-styrenesulfonic acid were delineated with reference to the blue dextran and the intermediate 4000 molecular weight poly(styrenesulfonate).

The foregoing experimental approach provided a means by which appreciable variations between the void and imbibed volumes of solvent in the column could be taken into account during any series of chromatographic studies. Thus the raw data (absorbance vs. time) was digitized and transformed, using a flexible Fortran IV program,[4] to plots of absorbance vs. retention volume, V_R, relative to that for *p*-nitrophenol (vide supra). The area under each curve was normalized by assuming a linear variation in flow rate between the initiation and completion of the particular run.

The narrow fractions selected for molecular weight determination from the profiles of the kraft and Organosolv lignin samples eluted with carbonate-free 0.10 M aqueous NaOH from Sephadex G75 (Pharmacia)[4] were not subsequently reeluted individually through the same chromatographic system. They were made up immediately for ultracentrifuge sedimentation equilibrium analysis so that data could be collected for the respective molecular weight determinations within 50 h of their isolation. The transformation of the raw elution profiles and the standardization of the retention volumes for the selected fractions were carried out as previously described.[4]

Ultracentrifuge Studies. The paucidisperse kraft lignin fractions isolated from the Sephadex G100/0.10 M aqueous NaOH system were made up to 0.10 M NaCl at pH 9.5 so as to contain less than 3.0×10^{-2} g L^{-1} lignin components. Their weight-average ($\bar{M}_w$) and *z*-average ($\bar{M}_z$) molecular weights were characterized by means of ultracentrifuge sedimentation equilibrium monitored with the photoelectric scanner in a Beckman Spinco Model E analytical instrument. The choice of pH conditions was dictated by the need to avoid risking damage to the rotor and Yphantis[10] six-channel centerpieces in which the sample and references solutions were held during these determinations. The experimental procedures adopted to allow data collection to the cell base in each solution sector have been reported previously.[4]

The fractions with $\bar{M}_w$ below 30 000 were scanned at two wavelengths (280 and 300 nm) and at three rotor speeds (14 000, 17 000, and 24 000 rpm) in the manner employed in earlier work.[4] Integration of the sedimentation curve for each fraction confirmed that, in the runs from which data were included in computing the respective $\bar{M}_w$ and $\bar{M}_z$, the total detectable mass of components was effectively conserved. This condition could not be realized, however, for the fractions with $\bar{M}_w$ above 60 000; data collected at the two wavelengths and a rotor speed of 11 000 rpm permitted calculation of only lower limits to the respective molecular weight averages. Attempts to reduce the rotor speed to 8000 rpm resulted in serious interference from convective effects.

The weight-average and *z*-average molecular weights for the fractions were calculated by using the equations of Lansing and Kraemer[11] from $M_{w,r}$ and $M_{z,r}$ assuming the component partial specific volume to be 0.653 cm^3 g^{-1} and independent of molecular weight.[12] The electronic components of the apparatus and the treatment of the raw data have been described elsewhere by Teller.[13] Although the *z*-average molecular weights were characterized by very large errors, the weight-average molecular weights appeared to be reliable.

The fractions selected from the Sephadex G75/0.10 M aqueous NaOH elution profiles of the kraft and Organosolv lignins were also made up to 0.10 M NaCl at pH 9.5 so as to contain less than 3.0×10^{-2} g L^{-1} lignin components. Each was scanned at two

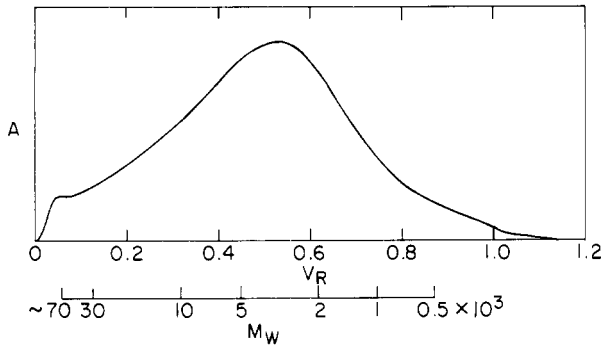

Figure 1. Molecular weight distribution of gymnosperm kraft lignin isolated from black liquor (Sephadex G100/0.10 M aqueous NaOH, monitored at 280 nm).

wavelengths (chosen between 280 and 365 nm) and at two rotor speeds (between 17 000 and 36 000 rpm). The weight-average molecular weights obtained from the four scans for a given sample, in which the detectable mass of components was conserved, were found to be consistent.[4]

Contribution from Polydispersity to Chromatographic Peak Variance. The form of a size-exclusion chromatographic profile generally depends both upon column dispersion and upon the polydispersity of the sample (the latter being determined by the distribution of solute conformations and configurations). The effect of column dispersion on symmetrical peaks close to Gaussian in shape can be estimated quite accurately from the approximate relationship[14] between the variance of the dispersion process (σ_{cd}^2) and the peak retention volume (V):

$$\sigma_{cd}^2 = (1.02/N)V^2$$

where N is the column plate count. In terms of the relative retention volume (V_R) with respect to the *p*-nitrophenol marker (vide supra), σ_{cd}^2 for the paucidisperse kraft lignin fractions is therefore proportional to $[V_R + V_0/(V_{pNP} - V_0)]$, where V_0 is the void volume and V_{pNP} is the retention volume for *p*-nitrophenol. The effect of column dispersion upon the peak width for each kraft lignin fraction was thereby determined by using the σ_{cd} (average of three values) for the monodisperse model dimer guaiacylglycerol β-(2-methoxyphenyl) ether. The contribution (in units of relative retention volume squared) from the actual polydispersity (σ_{pd}^2) to the overall peak variance (σ_{peak}^2) may then be deduced from $\sigma_{pd}^2 = \sigma_{peak}^2 - \sigma_{cd}^2$. In these calculations, σ_{peak} was taken to be equal to the peak width at half-height multiplied by 0.43.

Results and Discussion

Conformation of Kraft Lignin Components. Figure 1 depicts the profile of the gymnosperm kraft lignin isolated from an industrial black liquor upon elution from Sephadex G100 with 0.10 M aqueous carbonate-free NaOH. From this sample a set of narrow fractions was selected that spanned a range in molecular weight of approximately 2 decades. Containing roughly 0.5 g L^{-1} kraft lignin species, these were incubated in 0.10 M aqueous NaOH for ~1500 h, a period sufficient to allow virtually complete dissociation between the components to occur.[4] The associative/dissociative effects observed with complete kraft lignin samples under such solution conditions were found to be reversible.

The Sephadex G100/0.10 M aqueous NaOH elution profiles for each fraction thus prepared exhibited a sharp rise to the peak maximum while the extent of apparent tailing (due to dissociation) increased progressively with the average molecular weight. Paucidisperse fractions comprised of discrete kraft lignin components were secured from narrow regions about the respective peak maxima.

The component weight-average molecular weights, $\bar{M}_w$, for these paucidisperse kraft lignin fractions (Table I) were determined from ultracentrifuge sedimentation equilibrium studies of aqueous solutions appropriately made up to 0.10 M NaCl at a pH of 9.5 (see Experimental Section). The individual profiles observed upon eluting from Sephadex G100 with 0.10 M aqueous NaOH are shown in Figure 2. The peaks arising from the same size-exclusion chromatographic system for standard poly(styrenesulfonate) fractions are illustrated in Figure 3. While the poly(styrenesulfonates) are significantly more polydisperse than the kraft lignin fractions, the elution characteristics for both series of polymeric components approach ideal behavior: except in the highest molecular weight region, the chromatographic bands are close to Gaussian in shape. Peak skewness is generally expected to be more pronounced for high molecular weight components,[15,16] although its causes are not well understood. Such an effect is detectable in the peaks eluting near the excluded limit for the gel.

Table I
Dependence of $\bar{M}_w$ on V_R for Paucidisperse Kraft Lignin and Poly(styrenesulfonate) Fractions Eluted with 0.10 M Aqueous NaOH from Sephadex G100[a]

kraft lignin fractions[b]			poly(styrenesulfonate) fractions[e]		
fraction no.	V_R[c]	$\bar{M}_w$[d]	fraction no.	V_R[c]	$\bar{M}_w$[f]
0[g]	0.050	>139 000[h]			
1	0.052	>106 000[i]	1	0.066	31 000
2	0.061	⩾66 000[j]			
3	0.136	27 800	2	0.155	16 000
4	0.233	15 900			
5	0.351	8 050	3	0.292	6 500
6	0.420	5 600			
7	0.489	4 250	4	0.387	4 000
8	0.533	2 990			
9	0.592	2 290	5	0.561	1 600
10	0.653	1 680			
GAE[k]	0.947	321	pSSA[l]	0.970	184

[a] Using 70 × 2.5 cm analytical column. [b] Isolated from black liquor (see Experimental Section); elution profiles shown in Figure 2. [c] Relative retention volume with respect to that for *p*-nitrophenol. [d] Weight-average molecular weights determined by ultracentrifuge sedimentation equilibrium studies of solutions at pH 9.5, ionic strength 0.10 M; unless otherwise stated values are based on the complete subset of components. [e] Obtained from Pressure Chemical Co. and used without further purification or fractionation; elution profiles shown in Figure 3. [f] Weight-average molecular weights reported by Pressure Chemical Co. [g] Omitted for clarity from Figure 2. [h] 22% of components sedimented out at cell base during ultracentrifugation. [i] 14% of components sedimented out. [j] Value based on 96% of the components originally present. [k] Guaiacylglycerol β-(2-methoxyphenyl) ether. [l] *p*-Styrenesulfonic acid.

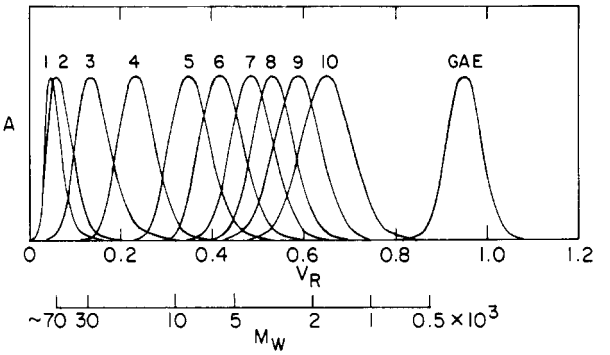

Figure 2. Elution profiles of kraft lignin fractions and model β-0-4-linked dimer listed in Table I (Sephadex G100/0.10 M aqueous NaOH, monitored at 280 nm). GAE = guaiacylglycerol β-(2-methoxyphenyl) ether.

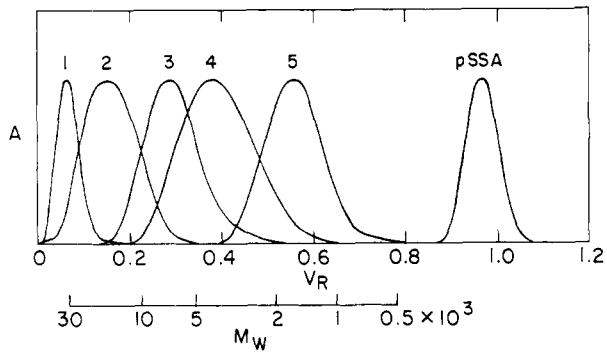

Figure 3. Elution profiles of poly(styrenesulfonate) fractions and monomer listed in Table I (Sephadex G100/0.10 M aqueous NaOH, monitored at 280 nm). pSSA = *p*-styrenesulfonic acid.

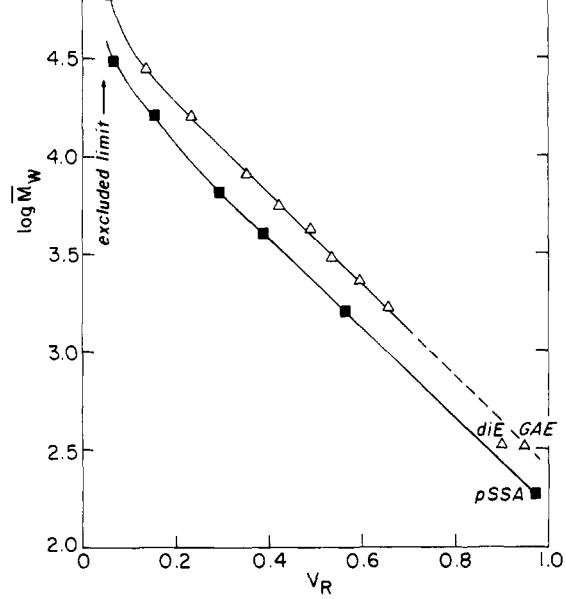

Figure 4. Semilogarithmic plots of weight-average molecular weight vs. relative retention volume for fractions of (△) gymnosperm kraft lignin and (■) poly(styrenesulfonates) eluted from Sephadex G100 with 0.10 M aqueous NaOH. GAE = guaiacylglycerol β-(2-methoxyphenyl) ether; diE = diisoeugenol; pSSA = *p*-styrenesulfonic acid.

The derived calibration curves for the kraft lignin and poly(styrenesulfonate) fractions are presented in Figure 4 as plots of log $\bar{M}_w$ vs. V_R, the relative column retention volume. The curve for the kraft lignin fractions is, to a high degree of accuracy, linear for $\bar{M}_w$ values extending to 25 000 (i.e., close to the excluded limit). Clearly, there is no indication in the analytical system at hand of spurious effects such as ion inclusion and ion exclusion[17] or adsorption of the lignin components onto the gel matrix.[18,19]

The value observed for the V_R of guaiacylglycerol β-(2-methoxyphenyl) ether places this compound, within experimental error, directly on the extrapolated calibration curve for the kraft lignin components (Figure 4); these components thus behave as though they are conformationally homologous with the lignin model β-0-4 linked dimer. (We are indebted to Mr. L. Hoo, College of Forest Resources, University of Washington, for kindly donating a sample of the model compound.) As expected, the poly(styrenesulfonates) can be seen in a similar way to be homologous with the *p*-styrenesulfonic acid monomer. The reliability of identifying by these means the effective conformational homology characteristic of a polymer series rests on the solute parameter that governs the entropically controlled[20] size-exclusion chromatographic process. In this respect, the hydrodynamic volume[21] of the solvated component is particularly sensitive to the conformation (and configuration) in the low molecular weight range. It is evident, for example, that diisoeugenol does not behave as though it were conformationally homologous with the discrete kraft lignin components (Figure 4).

Table II
Effect of Ionic Strength upon Relationship between Radius of Gyration and Molecular Weight for Poly(styrenesulfonates)[a]

ionic strength,[b] M	expansion factor[c]	molecular weight exponent[d]	no. of data points
0.5	2.5	0.38	4
0.1	3.1	0.47	5
0.05	3.3	0.44	4
0.01	3.6	0.69	4
0.005	3.7	0.55	3

[a] Determined from data at 25 °C obtained by light scattering[23] for poly(styrenesulfonate) fractions with $\bar{M}_w$ ranging between 3.9×10^5 and 2.34×10^6. [b] Maintained with NaCl in aqueous solution. [c] Ratio of radius of gyration, $\langle s^2 \rangle_z^{1/2}$, at given ionic strength to unperturbed radius under Θ solution conditions (3.1 M aqueous KCl) for poly(styrenesulfonate) fraction with $\bar{M}_w = 1.00 \times 10^6$. [d] Slope of linear regression of log $\langle s^2 \rangle_z^{1/2}$ on log $\bar{M}_w$; the results listed have been confined to those for which the correlation coefficient is better than 0.98. No attempt has been made to correct the radii to weight-average values.

In the region where both are linear, the plots of log $\bar{M}_w$ vs. V_R for the kraft lignin and poly(styrenesulfonate) fractions are characterized by slopes of −2.35 and −2.29, respectively. Differing by only 2.5%, the magnitudes of the slopes may be taken, within experimental error, to be identical. Thus, at any given retention volume in this range, the kraft lignin components exhibit molecular weights that are larger by a factor of 1.7 than those of the poly(styrenesulfonates). This finding has the important practical application that the profiles for sets of kraft lignin components eluted from cross-linked dextran gels with 0.10 M aqueous NaOH can be indirectly calibrated quite accurately by commercially available poly(styrenesulfonate) fractions.

In a homologous polymeric series, the molecular conformation will determine the magnitude of the slope of the size-exclusion chromatographic calibration curve for a given pore-size distribution in the column packing.[22] The functional dependence of the radius of gyration ($\langle s^2 \rangle_z^{1/2}$) on the molecular weight must therefore be very similar among both the kraft lignin and the poly(styrenesulfonate) components. This relationship has been deduced from light-scattering data published[23] for a set of poly(styrenesulfonate) fractions in aqueous solution at ionic strengths between 0.005 and 0.5 M.

The results summarized in Table II compare the empirical molecular weight exponent (x when $\langle s^2 \rangle_z^{1/2} \propto \bar{M}_w^x$) with the expansion factor relative to the unperturbed molecular dimension under Θ solution conditions. No attempt has been made to correct the *z*-average radii of gyration ($\langle s^2 \rangle_z^{1/2}$) to weight-average values, which would have been more appropriate for correlation with weight-average molecular weights ($\bar{M}_w$). However, the differences between the molecular weight exponents would not be altered thereby if all of the poly(styrenesulfonate) fractions have about the same degree of polydispersity.[24] Furthermore, the uncertainties associated with the reported

values of $\langle s^2 \rangle_z^{1/2}$ are so large as to reveal only the trend that would be expected in the variation of the molecular weight exponent with the expansion factor (Table II).

Nevertheless, the comparison with the data available for the poly(styrenesulfonates) gives a direct indication of the conformation adopted by the discrete kraft lignin components in 0.10 M aqueous NaOH. They evidently behave as appreciably expanded random coil molecules for which the relationship between radius of gyration and molecular weight is characterized by a molecular weight exponent of approximately 0.5. The effective expansion factor presumably has a value less than 3.1, namely, that observed at the same ionic strength for a $1.00 \times 10^6 \bar{M}_w$ poly(styrenesulfonate) fraction (Table II).[23]

Branching. The universal calibration concept for size-exclusion chromatography[25-27] confirms that the governing solute parameter is the hydrodynamic volume[21] when the process is entirely entropy controlled.[20] The ratio of the mean-square radii of gyration for branched to linear molecules in Θ solvents has been theoretically related to the number of randomly disposed (trifunctional and tetrafunctional) branch points in both monodisperse and polydisperse polymeric systems.[28] Although branching may not necessarily influence the hydrodynamic radius to the same extent as it affects the radius of gyration,[28,29] plots of log $\bar{M}_w$ vs. V_R for a series of polymer fractions with constant branching index will clearly exhibit curvature.[30] While the expansion factor may be larger for branched than for linear molecules in good solvents,[24] the evident linearity of the log $\bar{M}_w$ vs. V_R calibration curve for the paucidisperse kraft lignin fractions (Figure 4) is incompatible with a molecular weight independent branching index for the constituent components (namely, the case where the number of branch points is directly proportional to the molecular weight). Furthermore, the kraft lignin components behave as though they are conformationally homologous with the model dimer guaiacylglycerol β-(2-methoxyphenyl) ether (Figure 4; vide supra); thus even the case where the number of long-chain branch points does not vary with molecular weight can also reasonably be excluded. On the other hand, the results from the present work would not be sensitive to short-chain branching in kraft lignin components, the effect of which on size-exclusion chromatographic calibration curves is generally small and constant.[30,31]

Polydispersity. The chromatographic peaks of the kraft lignin fractions (2–10 in Figure 2) with $\bar{M}_w$ values of 66 000 and below are close to Gaussian in shape. An approximately logarithmic-normal distribution of the component subset comprising each would thus be anticipated. Although originally proposed to describe polymer samples containing very few low molecular weight species,[11] the logarithmic-normal distribution function is well suited for fractions prepared by size-exclusion chromatography because of the empirically observed semilogarithmic relationship between the molecular weight and retention volume. The component distribution in such a case is characterized by a polydispersity index that is given by[11]

$$\bar{M}_z/\bar{M}_w = \bar{M}_w/\bar{M}_n = \exp(\sigma_{pd}^2)$$

where σ_{pd}^2 is the variance of the distribution and $\bar{M}_z$, $\bar{M}_w$, and $\bar{M}_n$ are the z-average, weight-average, and number-average molecular weights, respectively.

The molecular weight, M_0, at the maximum value of dw/dM is given by $\bar{M}_w(\bar{M}_w/\bar{M}_z)^{1.5}$, where w and M are the weights and molecular weights, respectively, of any logarithmic-normally distributed set of conformationally homologous components. When the chromatographic peak for a fraction is located within the region where log M_0 is a linear function of V_R with slope D, $\bar{M}_z/\bar{M}_w$ is related to σ_{pd}^2 by

$$\log (\bar{M}_z/\bar{M}_w) = (\ln 10)D^2\sigma_{pd}^2$$

if σ_{pd} is expressed in units of relative retention volume. With the slope of the kraft lignin calibration curve (Figure 4) as the first approximation, iterations upon $\{\log \bar{M}_w - 1.5(\ln 10)D^2\sigma_{pd}^2\}$ allow calculation of successive values for D which rapidly converge to −2.38. Thus log $(\bar{M}_z/\bar{M}_w)$ would be expected to vary as $13.1\sigma_{pd}^2$.

Table III
Comparison of $\bar{M}_z/\bar{M}_w$ for Paucidisperse Kraft Lignin Fractions with the Contribution from Polydispersity to Chromatographic Peak Variance[a]

fraction no.	$\sigma_{cd}^2 \times 10^3$ [b]	$\sigma_{pd}^2 \times 10^3$ [c]	$\bar{M}_z/\bar{M}_w$ [d]
2	0.19	0.52	2.0 ± 0.3
3	0.32	0.97	1.4 ± 0.3
4	0.40	1.19	1.6 ± 0.3
5	0.55	1.42	1.5 ± 0.4
6	0.64	1.65	1.6 ± 0.2
7	0.73	1.37	2.7 ± 0.4
8	0.77	1.25	1.0 ± 0.2
9	0.87	1.67	2.1 ± 1.0
10	0.95	2.03	3.1 ± 0.7
GAE	1.38	(0.0)	(1.0)

[a] Ratios of z-average to weight-average molecular weights were deduced from ultracentrifuge sedimentation equilibrium studies of solutions at pH 9.5, ionic strength 0.10 M, while the chromatographic peak variance is that observed (Figure 2) during elution from Sephadex G100 with 0.10 M aqueous NaOH. [b] Contribution from column dispersion to peak variance (in units of relative retention volume squared) estimated from peak variance of guaiacylglycerol β-(2-methoxyphenyl) ether (Figure 2). [c] Contribution from polydispersity to peak variance (in units of relative retention volume squared) determined as described in Experimental Section. [d] Standard deviations for $\bar{M}_z/\bar{M}_w$ are those arising from the uncertainty in $\bar{M}_z$ values.

The chromatographic values deduced for σ_{pd}^2 of the kraft lignin fractions (see Experimental Section) are compared in Table III with the $\bar{M}_z/\bar{M}_w$ ratios determined by ultracentrifuge studies. The linear regression of log $(\bar{M}_z/\bar{M}_w)$ on σ_{pd}^2 is characterized by a correlation coefficient of 0.38. Its small magnitude is presumably caused primarily by the large errors associated with the values of $\bar{M}_z$. Since the correlation between log $(\bar{M}_z/\bar{M}_w)$ and σ_{pd}^2 cannot be taken to be significant, the data are appropriately summarized by ascribing to each fraction an average value of 1.9 ± 0.3 for $\bar{M}_z/\bar{M}_w$.

On the other hand, calculation of this ratio from the chromatographic peak variance using the above equation suggests a nearly constant value of 1.04 ± 0.01 for the $\bar{M}_z/\bar{M}_w$ of the fractions. (This is actually an upper limit to the ratio since no estimate has been included to account for the distribution of conformations for molecules with a given molecular weight and configuration.) Put another way, the expected contribution from the $\bar{M}_z/\bar{M}_w$ determined by ultracentrifuge studies to the chromatographic peak variance is an order of magnitude larger than the observed σ_{pd}^2 for the most polydisperse fraction will permit (on the basis of a logarithmic-normal distribution of conformationally homologous kraft lignin components).

The cause of the apparent dilemma may be deduced from the following considerations. Each kraft lignin fraction could be viewed as being comprised of a set of components with differing configurations but the same effective hydrodynamic volume. The ultracentrifuge results indicate that the molecular weight distribution, if

continuous, would be very broad. For example, the variance of a logarithmic-normal distribution in such a case would be ln (1.9/1.04); the implication would be that 68% of the molecules eluted at a particular retention volume are enclosed within a band extending from 0.055 to 0.735 log unit below the log $\bar{M}_w$ vs. V_R calibration curve for the kraft lignin fractions!

Whatever the actual form adopted by the molecular weight distribution of a particular fraction, the molecules in the lower molecular weight range would tend conformationally toward rigid rods, while those with higher molecular weights would approach a spherical shape. In the limit, calibration curves for rodlike and spherelike solutes would exhibit slopes that in magnitude are, respectively, 0.5 and 1.5 times that for random coil molecules.[22] The polydispersity of the kraft lignin fractions would thus be expected to increase significantly with molecular weight. There is, however, no indication of such an effect (Tables I and III).

It is reasonably certain, therefore, that the magnitude (1.9 ± 0.3) of $\bar{M}_z/\bar{M}_w$ for the kraft lignin fractions results from limited association between the components under the solution conditions at pH 9.5 that were imposed for ultracentrifuge sedimentation equilibrium studies (see Experimental Section). As an example compatible with the observed value for $\bar{M}_z/\bar{M}_w$, a 1.46×10^{-3} molar ratio of $10M_i$ to M_i molecular weight species in a two-component mixture would cause $\bar{M}_w$ to be 13% larger than M_i. In this connection, the finding of a molecular weight invariant $\bar{M}_z/\bar{M}_w$ for the kraft lignin fractions (Table III) is in accord with the evident linearity over a broad range of the calibration curve (Figure 4). Taken together, these observations imply that the degree of association within each paucidisperse set of components is both small in magnitude and independent of average molecular weight.

Configuration of Associated Complexes. Discrete lignin components exhibit a range of conformations in solution that will be distinct from the configurations of the complexes formed through association between them. An important aspect of this relationship is revealed by the properties of lignin monolayers on a water surface.[32] Estimates of the monomolecular film thickness have been obtained by means of a Langmuir trough for selected kraft, milled-wood, and dioxane lignin samples with apparent weight-average molecular weights ranging from 2900 to 85000. Using the area extrapolated from the linear portion of each force–area isotherm to zero pressure, Luner and Kempf found the film thickness (1.6 ± 0.2 nm) to be independent of apparent molecular weight.[32]

The lignin monolayers were spread from ethanol/1,2-dichloroethane solutions,[32] conditions strongly favoring association between the components.[3] Providing that the monomolecular films were not primarily comprised of low molecular weight components preferentially extracted from the complete lignin samples, these results are quite informative. It has been proposed that they imply a flat "disklike" configuration for the species present in the monolayers.[33]

Support for these arguments has been forthcoming from photomicrographic images obtained with an electron microscope of the lignin sulfonate macromolecules in a relatively paucidisperse high molecular weight preparation: the dimensions suggested a disklike shape with an average thickness of about 2 nm for the components present.[34] Since there has been only one unconfirmed report of associative interactions between lignin sulfonate molecules,[1] however, the shape of these strongly polyionic species may only have an indirect bearing upon the configuration of

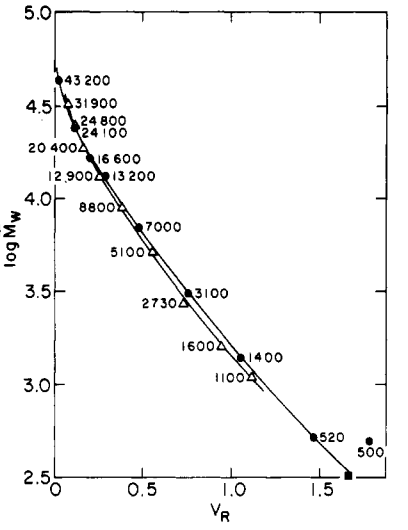

Figure 5. Calibration curves for (△) gymnosperm kraft lignin and (●) red alder Organosolv lignin samples eluted from Sephadex G75 with 0.10 M aqueous NaOH. (■) Guaiacylglycerol β-(2-methoxyphenyl) ether.

kraft and Organosolv lignin complexes. Nevertheless, several lignin derivatives exhibit solution properties that are intermediate between those expected for a non-free-draining coil and an Einstein sphere.[35] It has been pointed out that these observations are consistent with a flexible disklike conformation for the various soluble lignin species.[33,34]

During the present work, the kraft lignin fractions that had been preparatively eluted with 0.10 M aqueous NaOH through Sephadex G100 were incubated for periods sufficient to allow virtually complete dissociation between the components to occur (see Experimental Section). Qualitatively, the relative proportions of associated complexes originally present were discernible from the individual profiles subsequently described by the Sephadex G100/0.10 M aqueous NaOH chromatographic system: the extent of tailing became progressively more marked with increasing molecular weight (vide supra).

The dependence of radius of gyration upon molecular weight for any homologous series of associated complexes would be expected to be intermediate between those for random coil and spherical configurations. These complexes contribute in greater numbers to the higher molecular weight fractions of lignin samples that have not been adequately exposed to solution conditions favoring dissociation. Thus appreciable curvature should be apparent in plots of log $\bar{M}_w$ vs. V_R for lignins containing significant proportions of associated complexes.

The anticipated effect is illustrated in Figure 5 not only for the kraft lignin sample but also for an Organosolv lignin preparation isolated from red alder. Here only 50 h have elapsed between fractionation and attainment of equilibrium in the ultracentrifuge (see Experimental Section).[4] These calibration curves pertain to profiles obtained by eluting with 0.10 M aqueous NaOH from Sephadex G75, a gel possessing a lower excluded limit than Sephadex G100. Nevertheless, the plots are clearly curved throughout the accessible molecular weight range. It seems, therefore, that the true calibration curve for a lignin sample will depend appreciably upon the degree of association between the components present.

Conclusions

Considered, then, in the light of the lignin monolayer studies,[32] the results from the present work support a flexible lamellar configuration[33,34] for associated lignin

complexes, while the discrete components behave in aqueous alkaline solution as appreciably expanded random coil molecules. The intermolecular associative effects are apparently governed by nonbonded orbital interactions,[4] presumably between the aromatic moieties in the components. On the other hand, the overall rates of association are determined by the conformational changes that lead to component conformations which are complementary to one another.[4] A thickness of 1.6 nm for monomolecular lignin films[32] would certainly accommodate the requirement for a perpendicular disposition of the benzene rings with respect to the surface of the associated complex. Consistent with this working hypothesis is the evident lack of a hydrodynamic effect arising from long-chain branching: such a factor in the individual components would have rendered associated complexes with the proposed configuration difficult to envisage. A more detailed understanding of the associative mechanism between the components should serve to reduce the apparent complexity that presently typifies the physicochemical properties of byproduct lignins.

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Effect of Polystyrene Molecular Weight on the Carbon Dioxide Sorption Isotherm

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ABSTRACT: Sorption isotherms for CO_2 at various temperatures were measured for several nearly monodisperse polystyrenes with molecular weights ranging from 3600 to 850 000 and for one commercial polydisperse polystyrene. Extent of sorption decreased with increasing temperature but increased as the molecular weight increased. The results were interpreted in terms of the dual-sorption model. The Langmuir capacity term was observed to increase with molecular weight of the polystyrene because of the corresponding increase in the glass transition. A quantitative analysis of this effect is presented.

Introduction

Information about the solubility of solute molecules in polymers is essential for quantifying transport processes such as permeation. When the solubility is quite small, as for gases, it has been common practice to infer this information from transport measurements, owing to the difficulties of direct measurement. For simple systems, the Henry's law solubility coefficient can be computed from experimentally observed diffusion time lags and the permeability coefficient as has been the practice for gases in polymers above their glass transition temperature. However, when the mechanism for sorption and transport deviates from simple linear models, as occurs in glassy polymers,[1] inference of sorption isotherms from transport observations becomes less reliable and direct measurement is essential. The recent results reported by Toi et al.[2]

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