for preparing the sample and acknowledge helpful discussions with Drs. T. Russell and P. Cotts concerning sample preparation and characterization. Dr. M. Mantler, visiting scientist from Vienna Technical University, made the energy-dispersive X-ray measurements and G. S. Lim aided in the diffractometer runs. N. Takahashi thanks IBM World Trade of Japan for providing a postdoctoral fellowship.

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# Lignin. 20. Associative Interactions between Kraft Lignin Components†

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ABSTRACT: The profiles described by eluting gymnosperm kraft lignins from dextran gels with 0.10 M aqueous NaOH represent effective molecular weight distributions that approach those for the discrete components. Under aqueous conditions between pH 13 and 14, dissociation of kraft lignin complexes occurs in dilute solution ($\sim$0.5 g $L^{-1}$), while a marked tendency for kraft lignin components to associate prevails at higher concentrations ($\sim$20–150 g $L^{-1}$). These processes are, furthermore, reversible. The apparent molecular weight distributions of kraft lignins are affected by the presence of counterions and zwitterions in alkaline solution and are remarkably sensitive to the method employed for their isolation. The relative ratios of kraft lignin components with molecular weights below 3500 do not, however, vary with the degree of association for the sample as a whole. The relationship between the overall weight-average and number-average molecular weights indicates that the ensemble average product of the molecular weights of interacting species remains constant during association in aqueous alkaline solution. This implies that the associative processes occurring within kraft lignin samples are stoichiometrically constrained: each of the associated complexes possesses a locus which is respectively complementary to only one type of component.

## Introduction

The first exploratory investigation revealing that lignin components tend to associate with one another was published 20 years ago.[1] However, the appearance three years later of a second article[2] demonstrating the existence of such phenomena prompted no further studies of the effect for more than a decade. Thus the field of lignin chemistry was beset by a conceptual deficiency at a relatively fundamental level: reliable methods for determining the molecular weight distributions of lignin samples were only recognized quite recently.[3]

In solutions without dissolved electrolytes, the apparent molecular weight distributions of gymnosperm kraft lignins

† 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 paper was also mainly written.

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extend to molecular weights 10000 times larger than those of the discrete components.[3] Moreover, rather than being continuous, these distributions are multimodal in form; the associated kraft lignin complexes therefore must somehow be well-defined. In aqueous alkaline solution, the associative processes occurring within angiosperm Organosolv lignin samples are not random: they are dominated by preferential interactions between the lower and higher molecular weight subsets of species.[4]

Discrete kraft lignin components adopt appreciably expanded random coil conformations in aqueous alkaline solution.[5] The associative phenomenon is characterized by two kinetically distinguishable steps.[4] The slower of the two is rate determining under solution conditions where lignin components are polyanionic; it presumably reflects conformational changes that lead to component conformations compatible with subsequently rapid association.[5]

The present work was directed toward developing a more complete understanding of the associative behavior exhibited by kraft lignin components. The apparent molecular weight distributions of kraft lignins have been found to be strongly dependent both upon isolation procedure and upon incubation conditions prior to size-exclusion chromatographic fractionation. More specifically, from the mode of association in aqueous alkaline solution, it has become evident that associated kraft lignin complexes may be topologically regular.

## Experimental Section

**Kraft Lignin.** The gymnosperm kraft lignin was isolated from kraft black liquor donated by the Weyerhaeuser Co. from their mill at Longview, WA, which utilizes mainly Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) wood. Diluted kraft black liquor (500 mL containing 120 g $L^{-1}$ dissolved solids after filtration through a VWR crepe white paper to remove residual fibrous material) was acidified with 0.5 M aqueous $H_2SO_4$ (0.5 mL $min^{-1}$) to pH 2.5. Most of the resulting solution (containing suspended solids) was centrifuged, and the precipitate was washed three times by resuspending in aqueous solution at pH 2.5 and centrifuging; the sample was then redissolved in the minimum volume of aqueous solution at pH 8.5 and freeze-dried therefrom. For comparative purposes, the kraft lignin was filtered from the small remaining portion of acidified black liquor and similarly washed three times before final filtration and air-drying; only 80% of the solid isolated by these means could be redissolved in aqueous solution at pH 8.5 and freeze-dried.

**Fractional Precipitation of Kraft Lignin.** Freshly diluted kraft black liquor (800 mL containing 170 g $L^{-1}$ dissolved solids after filtration through a VWR crepe white paper) was progressively acidified with 0.5 M aqueous $H_2SO_4$ at a rate of 0.5 mL $min^{-1}$. The solution (containing suspended solids) was successively centrifuged at pHs 9.5, 8.5, 7.5, 6.5, 5.5, and 4.0 until separation of the respective precipitates was complete; no further precipitation below pH 4.0 was observed under these circumstances. Each was redissolved in the minimum volume of aqueous solution at pH 8.5 and freeze-dried therefrom. Comparative experiments revealed that the yields, but not the apparent molecular weight distributions, of the kraft lignin fractions thus obtained at the various pH values exhibited a marked dependence upon the incubation time of the diluted black liquor prior to its acidification.

**Organosolv Lignin.** The Organosolv lignin from red alder (*Alnus rubra*), used as an auxiliary calibrant for these studies, was donated by Professor K. V. Sarkanen and co-workers, College of Forest Resources, University of Washington.[4]

**Size-Exclusion Chromatography.** A 70 × 2.5 cm (cylindrical) column was found to be adequate for routine analysis under the conditions employed in the present work. Column dispersion for components with molecular weight ~500 was generally no more than 8% of the relative column retention volume, $V_R$, when the 0.10 M aqueous NaOH eluant solutions were prepared so as to be free of carbonate. A single-beam LKB 8300A Uvicord II photometer (set at 254 or 280 nm) or double-beam ISCO UA-5 monitor with Type 6 optical unit (set at 280 nm) was used as a detector at the column outlet. The raw data (transmittance or absorbance vs. time) were digitized and transformed to elution profiles represented by plots of absorbance vs. relative retention volume, $V_R$, as previously reported.[4]

**Ultracentrifuge Studies.** Paucidisperse kraft lignin fractions selected from the Sephadex G75/0.10 M aqueous NaOH elution profiles were made up to 0.10 M NaCl at pH 9.5 so as to contain less than $3.0 \times 10^{-2}$ g $L^{-1}$ lignin components. The weight-average molecular weights ($\bar{M}_w$) were determined 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 An-F rotor and three Yphantis[6] six-channel centerpiece assemblies in which nine sample and reference solutions were simultaneously held during these determinations.

The weight-average molecular weights for the fractions were calculated from $M_{w,r}$ using the formalism of Lansing and Kraemer;[7] the component partial specific volume was taken to be 0.653 $cm^3$ $g^{-1}$ and independent of molecular weight.[8] The electronic components of the apparatus and the treatment of the raw data have been described elsewhere by Teller.[9]

The fractions were scanned at two wavelengths (310 and 365 nm) and at two rotor speeds (24000 and 34000 rpm). Integration of the sedimentation curve for each fraction confirmed that, in the runs from which data were included in computing the respective $\bar{M}_w$, the total detectable mass of components was effectively conserved. The weight-average molecular weights obtained from these scans were indeed found to be consistent with one another. The experimental procedures adopted to allow data collection to the cell base in each solution sector have been previously reported.[4]

The elution profiles from the Sephadex G100/0.10 M aqueous NaOH system, on the other hand, were calibrated by appropriate correlation with data published in the preceding paper of this series.[5]

**Apparent Average Molecular Weights.** The apparent weight-average and number-average molecular weights, $\bar{M}_w$ and $\bar{M}_n$, for the complete kraft lignin samples were directly calculated from the calibrated elution profiles assuming the absorptivity at 280 nm to be independent of molecular weight for all of the species present. This assumption is only approximately true for the components with molecular weights below ~1000, but the introduction of a systematic error hereby affects only the apparent stoichiometry of the associative processes being investigated: since the areas of the profiles for a given sample were, within experimental error, independent of solution history, the *relative* variations in $\bar{M}_w$ and $\bar{M}_n$ are quantitatively reliable.

## Results and Discussion

**Calibration of Size-Exclusion Chromatographic Elution Profiles.** Previous work[3,5] has established that the profiles described by eluting kraft lignins from crosslinked dextran gels with 0.10 M aqueous carbonate-free NaOH represent molecular weight distributions which approach those for the discrete components. Figure 1 depicts the absolute molecular weight calibration curve that characterizes the elution profile, revealed by fractionation through Sephadex G75, of the gymnosperm kraft lignin isolated from a stock industrial black liquor. This calibration curve has been deduced by ultracentrifuge sedimentation equilibrium analysis of the component weight-average molecular weights, $\bar{M}_w$, of paucidisperse fractions that span nearly the full range of relative retention volume, $V_R$, contained within the profile. The resulting plot of log $\bar{M}_w$ vs. $V_R$ for the kraft lignin sample is compared in Figure 1 with the calibration curve for a red alder Organosolv lignin described in an earlier report.[4] Despite a difference in the values demarcating the $V_R$ scale, the *relative* placement of the two curves between the axes is, within experimental error, identical with that previously observed using a different batch of Sephadex G75 gel.[4] The reproducibility inherent in the analytical technique

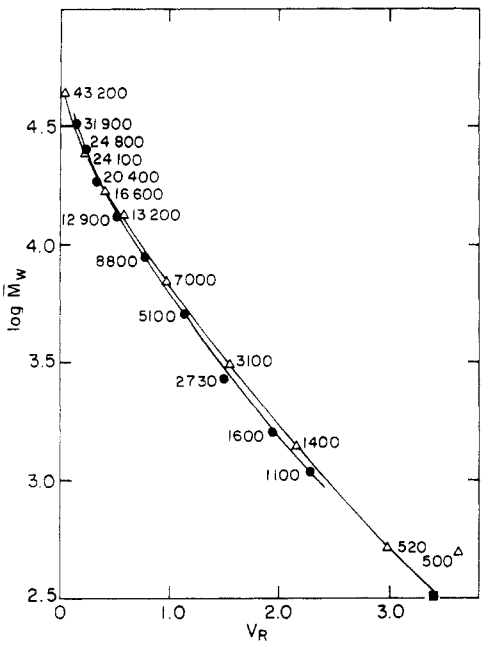



**Figure 1.** Calibration curves for (●) gymnosperm kraft lignin and (△) red alder Organosolv lignin samples eluted from Sephadex G75 with 0.10 M aqueous NaOH. (■) Guaiacylglycerol β-(2-methoxyphenyl) ether.

at hand is further confirmed by the $V_R$ value experimentally determined for guaiacylglycerol β-(2-methoxyphenyl) ether which places this dimeric lignin model compound, as before,[4] directly on the calibration curve.

The fractions selected for the ultracentrifuge sedimentation equilibrium studies were made up to 0.10 M NaCl at a pH of 9.5 so as to contain less than $3 \times 10^{-2}$ g $L^{-1}$ lignin components. Imposed in order to avoid damaging the ultracentrifuge rotor and cell housings, these solution conditions embody the same ionic strength as, but a pH over 3 units lower than, the respective parameters prevailing during size-exclusion chromatographic fractionation. A detailed analysis of the ratios of *z*-average to weight-average molecular weights for paucidisperse fractions of discrete kraft lignin components has suggested that some intermolecular association does occur under these conditions.[5] The observations imply that the degree of association within each paucidisperse component subset is, fortunately, both small in magnitude and independent of average molecular weight.[5] It is nonetheless evident that the calibration placed upon the presently reported results should be taken as an upper limit to the true molecular weights.

**Effect of Isolation Procedure on Kraft Lignin Molecular Weight Distribution.** The apparent molecular weight distribution of gymnosperm kraft lignin is remarkably sensitive to the method employed for isolating the sample from black liquor. The straightforward acidification of an industrial black liquor is cited here as an illustrative example. After the stock solution had been carefully acidified to pH 2.5 (see Experimental Section), centrifugation of the kraft lignin precipitate and subsequent freeze-drying from aqueous solution at pH 8.5 yielded a sample for which the molecular weight distribution is described by profile 1 in Figure 2. However, when the precipitate was instead filtered from acidified black liquor and air-dried, only a portion (constituting, in the present work about 80%) of the resulting solid could be redissolved in aqueous solution at pH 8.5 and freeze-dried therefrom; the molecular weight distribution for this portion is represented by profile 2 in Figure 2. The other

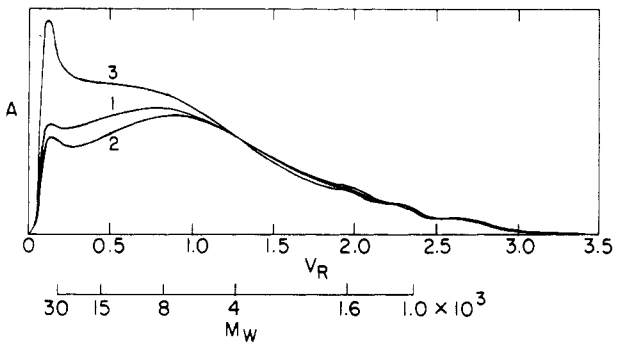



**Figure 2.** Variations in kraft lignin molecular weight distribution arising from method of isolating sample precipitated from black liquor at pH 2.5 (Sephadex G75/0.10 M aqueous NaOH, monitored at 254 nm): after (1) centrifugation and subsequent freeze-drying from aqueous solution at pH 8.5; following filtration and air-drying, portions (2) soluble in (and freeze-dried from) and (3) insoluble in aqueous solution at pH 8.5.

20% of the filtered sample remained in the form of granular particles and was presumably derived from locally moister regions in the *originally homogeneous* precipitate; upon redissolution in 0.10 M aqueous NaOH, this fraction revealed the component molecular weight distribution depicted by profile 3 in Figure 2.

Despite their common origins, these three kraft lignin samples differ markedly from one another in the contribution that the higher molecular weight species make to their respective molecular weight distributions. Indeed an appropriately weighted sum of profiles 2 and 3 shows that the kraft lignin as a whole contained a larger proportion of high molecular weight components after filtering and air-drying than after centrifuging the precipitate from acidified black liquor. It should be pointed out that, upon prolonged incubation at low concentrations (0.5 g $L^{-1}$) in 0.10 M aqueous NaOH (vide infra), the elution profiles of all three samples approached the *same* form; the contribution from species with molecular weights around 30 000 to this limiting molecular weight distribution was over 5 times smaller than to any of those depicted in Figure 2. Clearly, then, protracted air-drying of the kraft lignin precipitate can significantly affect the degree of association between the components in the sample; these observations could not have arisen from covalent chemical changes such as oxidative coupling between phenolic moieties.[10,11]

The elution profiles in Figure 2 have been scaled[12] so as to confer the same value upon the areas under the respective portions of these curves below $\bar{M}_w = 3500$. It has hereby become evident that while the proportions of higher molecular weight species exhibit substantial variations, the oligomeric components of the subset with molecular weight below ~3500 in the three samples remain, within experimental error, in the same relative ratios. Thus the components comprising this lower molecular weight subset must associate in constant (roughly 1:1) proportions with respect to each other. Such behavior would, a priori, be surprising for a subset of oligomers which have degrees of polymerization greater than the critical chain length for associated complex formation:[13] the equilibrium constant for association would be expected to increase rapidly with molecular weight so that preferential association of the higher molecular weight components should be strongly favored.[14]

**Association in Aqueous Alkaline Solution.** The extent to which association can occur between kraft lignin components in aqueous alkaline solution was found to be far greater than that which might be achieved during air-drying of precipitated samples. These findings were

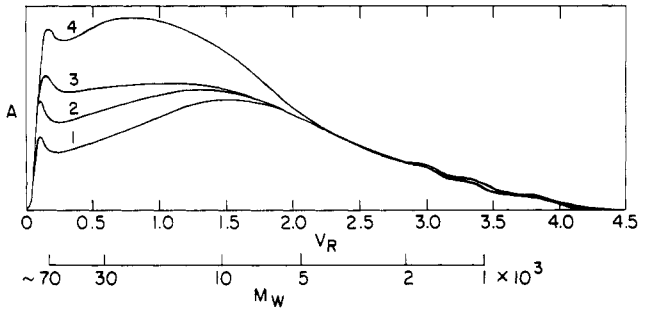



**Figure 3.** Combined effect on kraft lignin molecular weight distribution resulting from isolation method and incubation conditions prior to size-exclusion chromatography (Sephadex G100/0.10 M aqueous NaOH, monitored at 254 nm): for sample isolated by centrifugation of precipitate and subsequent freeze-drying from aqueous solution at pH 8.5, elution profiles after (1) 0.5 h and (2) 24 h at 20 g $L^{-1}$ in 0.10 M aqueous NaOH; following filtration and air-drying, profiles for (3) portion soluble in (and freeze-dried from) aqueous solution at pH 8.5, after 48 h at 20 g $L^{-1}$ in 0.10 M aqueous NaOH, and (4) portion insoluble in aqueous solution at pH 8.5, after 72 h at 150 g $L^{-1}$ in 1.0 M aqueous NaOH.

conveniently documented by monitoring solutions of the kraft lignin preparations isolated by the acidification of black liquor (vide supra). The portion of the filtered air-dried precipitate that was soluble at pH 8.5 exhibited the effects of significant association during incubation at 20 g $L^{-1}$ in 0.10 M aqueous NaOH: after 48 h its molecular weight distribution revealed a larger proportion of higher molecular weight species (profile 3, Figure 3) than that describing the sample centrifuged from acidified black liquor and fractionated after incubation for 24 h (profile 2, Figure 3).

The degree of association attainable between kraft lignin components at concentrations of 150 g $L^{-1}$ in 1.0 M aqueous NaOH was considerably greater. This is illustrated in Figure 3 for the fraction insoluble at pH 8.5 of the filtered air-dried precipitate after it had been incubated under these conditions for 72 h (profile 4); compared with the kraft lignin isolated from black liquor directly (profile 1), the molecular weight distribution here (profile 4) formally embodies a 1.7-fold increase in the degree of association[15] for the sample.

In the present work, the formal degree of dissociation was taken to be the ratio of the number of components with molecular weight below 3500 to that after complete dissociation of the kraft lignin sample (vide infra: "Dissociation in Aqueous Alkaline Solution"). The justification for this attribution will emerge from a detailed analysis of the mode of the dissociative process exhibited by kraft lignin under these conditions (vide infra: "Mode of Association").

The preceding results are remarkable in view of the polyanionic character of kraft lignin components in aqueous alkaline solution. It is unlikely that the intermolecular interactions could be favorable under these conditions were not the associative processes accompanied by proton abstraction from solution:[4] the negative charge density on the resulting complexes would otherwise be prohibitively large.

As a precaution, for the duration of these associative changes care was exercised in excluding atmospheric oxygen from contact with the aqueous alkaline solutions. Nevertheless, comparative studies indicated that exposure to air did not have a detectable effect upon the form of the kraft lignin molecular weight distributions which were developed during incubation under identical solution conditions.

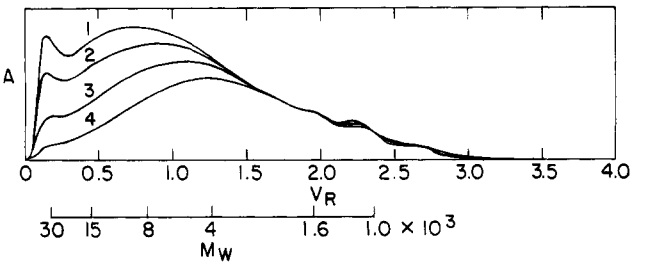



**Figure 4.** Dissociation of kraft lignin components in 0.10 M aqueous NaOH after (1) 0.8 h at 1.0 g $L^{-1}$, (2) 98 h at 1.0 g $L^{-1}$, (3) 122 h at 1.0 g $L^{-1}$, then 287 h at 0.50 g $L^{-1}$, and (4) 122 h at 1.0 g $L^{-1}$, then 1610 h at 0.50 g $L^{-1}$ (Sephadex G75/0.10 M aqueous NaOH elution profiles monitored at 280 nm).

**Table I**
**Effect of Dissociation on Apparent $\bar{M}_w$ and $\bar{M}_n$ of Kraft Lignin[a] in 0.10 M Aqueous NaOH**

| concn, g $L^{-1}$ | time, h | concn, g $L^{-1}$ | time, h | $\bar{M}_w$ | $\bar{M}_n$ |
|---|---|---|---|---|---|
| 1.0 | 0.8 | | | 9680 | 3490 |
| 1.0 | 98 | | | 8430 | 3170 |
| 1.0 | 388 | | | 6990 | 2930 |
| 1.0 | 122 | 0.5 | 287 | 6860 | 2870 |
| 1.0 | 122 | 0.5 | 1612 | 5390 | 2600 |
| 13[b] | 0.8 | | | 7820 | 3050 |

[a] Isolated from an industrial softwood kraft black liquor (see Experimental Section). [b] In 0.10 M aqueous NaOH containing 0.10 M betaine.

The areas of the elution profiles in Figure 3 were, within experimental error, independent of both the isolation procedure and the preincubation conditions to which the sample had been subjected. Appropriate scaling[12] of these profiles has revealed that the relative ratios of components in the subset with molecular weight below 3500 remain, within experimental error, constant despite the large differences in the degree of association for the kraft lignin as a whole.

**Dissociation in Aqueous Alkaline Solution.** The findings summarized above demonstrate that the apparent molecular weight distribution of kraft lignin can be significantly altered both during drying of precipitated samples and during standing in aqueous alkaline solution. Thus the Sephadex G75/0.10 M aqueous NaOH elution profile for a kraft lignin preparation directly isolated from black liquor is unlikely to be identical with the molecular weight distribution for the discrete components (profile 1, Figure 4). Indeed incubation of the sample at concentrations of 1.0 and 0.5 g $L^{-1}$ in 0.10 M aqueous NaOH was found to elicit marked dissociation for the higher molecular weight complexes (Figure 4). The process, which reached completion within 1700 h under these conditions, was accompanied by a 5.4-fold reduction in the proportion of species with molecular weights greater than 30 000. As shown in Table I, the apparent weight-average molecular weight of the sample concomitantly decreased (by a factor of 1.8) to 5390, a value which most closely approximates that for the discrete kraft lignin components.

The area (per unit sample weight) of each elution profile was found to be, within experimental error, independent of prior incubation conditions. The relative scaling[12] adopted for the presentation of these profiles in Figure 4 demonstrates that the components in the subset with molecular weight below 3500 are released in constant proportions to one another during the course of the dissociative process. In as far as the equilibrium molecular weight distribution in 0.10 M aqueous NaOH at a 0.5 g $L^{-1}$ sample concentration (profile 4, Figure 4) approaches that for the discrete components, the formal degree of associ-

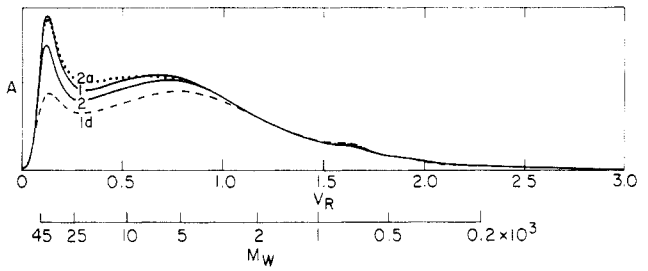



**Figure 5.** Reversibility of associative/dissociative processes exemplified for an Indulin AT sample (Sephadex G75/0.10 M aqueous NaOH monitored at 280 nm): (1) precipitate isolated by acidification to pH 3.0 after 40 h at 150 g $L^{-1}$ in 1.0 M aqueous NaOH and (1d) subsequent dissociation during 630 h at 0.50 g $L^{-1}$ in 0.10 M aqueous NaOH; (2) precipitate isolated by acidification to pH 3.0 after 42 h at 0.50 g $L^{-1}$ in 1.0 M aqueous NaOH and (2a) subsequent association during 66 h at 120 g $L^{-1}$ in 1.0 M aqueous NaOH.

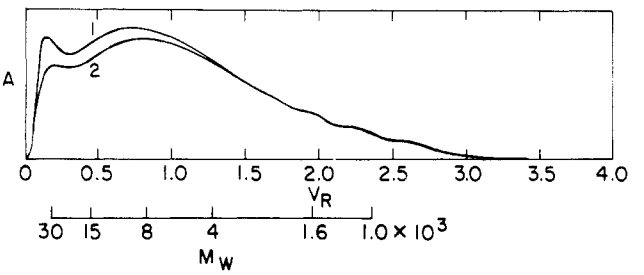



**Figure 6.** Counterion effects on molecular weight distribution of kraft lignin: elution from Sephadex G75 with (1) 0.10 M aqueous NaOH and (2) 0.10 M aqueous LiOH (monitored at 280 nm).

ation[15] for the kraft lignin in black liquor was 0.45 (profile 1, Figure 4). During incubation at a 150 g $L^{-1}$ sample concentration of kraft lignin in 1.0 M aqueous NaOH, the formal degree of association reached 0.70 in a 72-h period (profile 4, Figure 3). It is quite striking that the relative ratios[12] of the components comprising the subset with molecular weight below 3500 remain unaffected by the wide variations in the overall degree of association for the sample. Preferential association of the higher molecular weight oligomers in the subset would normally have been anticipated.[14] Evidently certain stoichiometric constraints act in some manner upon the associative processes in which these components participate.

**Reversibility of Associative/Dissociative Effects.** The behavior of kraft lignin samples at varying concentrations in aqueous alkaline solution does not arise from covalent chemical changes such as oxidative coupling of phenols[10,11] or reactions of the retroaldol type:[16] the associative/dissociative phenomena are fully reversible.

Solution conditions can readily be devised which consecutively promote processes of association and dissociation, or dissociation and association, between kraft lignin components. The results of such sequential processes are illustrated in Figure 5 for a commercially available (southern pine) kraft lignin preparation (Indulin AT). After incubation for 40 h at 150 g $L^{-1}$ in 1.0 M aqueous NaOH, the apparent molecular weight distribution of the kraft lignin sample was represented by profile 1, Figure 5; subsequent dissociation during a 630-h period at 0.50 g $L^{-1}$ in 0.10 M aqueous NaOH effected a marked reduction in the proportions of high molecular weight species as shown by profile 1d, Figure 5. On the other hand, following incubation for 42 h at 0.50 g $L^{-1}$ in 1.0 M aqueous NaOH, an apparent molecular weight distribution equivalent to profile 2, Figure 5, was observed; subsequent association during 66 h at 120 g $L^{-1}$ in 1.0 M aqueous NaOH caused a substantial increase in the proportion of high molecular weight species, evident in profile 2a, Figure 5.

Although the times allotted to incubation under the foregoing conditions were not sufficient to achieve equilibrium between the interacting kraft lignin components, the reversibility of the associative/dissociative phenomena has been convincingly demonstrated. Conforming with the pattern already established, the components of the lower molecular weight oligomeric subset maintain the same relative ratios[12] to one another despite marked variations in the overall degree of association.

After each incubation period and before size-exclusion chromatographic fractionation, the kraft lignin samples were precipitated from solution by acidifying to pH 3.0; they were then redissolved in the minimum volume of aqueous solution at pH 8.5 and freeze-dried. The effective molecular weight distributions characterizing these preparations (Figure 5) underscore an important distinction between the reversible associative interactions among lignin components and the (nonreversible) aggregative processes[17] leading to precipitation from solution: lignin samples can readily be isolated which differ only in degree of association in the solid state.

**Counterion and Zwitterion Effects.** It is apparent that the molecular weight distributions generated by eluting kraft lignins with 0.10 M aqueous NaOH from cross-linked dextran gels are not generally identical with those for the discrete components. The samples exhibit varying degrees of association that depend upon the conditions prevailing during their isolation and/or preincubation in solution prior to size-exclusion chromatographic fractionation. It is unlikely that associated kraft lignin complexes could remain intact in aqueous alkaline solution were not their formation from the individual polyanionic components accompanied by proton abstraction from solution: the negative charge density on the resulting species would otherwise be prohibitively large. It would thus be expected that any means of blocking proton uptake will significantly reduce the degree of association in alkaline solution. Either counterions or zwitterions could act in such a manner.

Recent work has shown that the presence of LiCl in the eluant DMF facilitates the dissociation of kraft lignin (Indulin) components during size-exclusion chromatographic fractionation from octyl-Sepharose CL-4B.[3] Indeed, the weight-average molecular weights for the same kraft lignin fractions calculated from octyl-Sepharose CL-4B/0.10 M LiCl–DMF and Sephadex G75/0.10 M aqueous NaOH elution profiles were found to be quite similar.[3] In order to clarify to what extent site binding by the lithium ion may contribute to the dissociative effects in DMF, the elution of kraft lignin samples from Sephadex G75 with 0.10 M aqueous LiOH was investigated. As shown in Figure 6, the elution profile was attenuated in the higher molecular weight region compared to when 0.10 M aqueous NaOH was used as eluant.

It is unlikely that these differences in degree of association for kraft lignin arise from territorial binding of condensed counterions[18] if the reported frequency (~0.45 per monomer residue[19]) of phenolic groups on the components is representative throughout the molecular weight range. The $^{23}Na$ nuclear magnetic relaxation rate in poly(acrylic acid) solutions increases rapidly with degree of neutralization above 0.35 but remains effectively constant at polyion charge densities less than the critical value.[20] Even in polyphosphate solutions[21] the $^{23}Na$ nuclear magnetic relaxation rate becomes independent of chain length only at degrees of polymerization greater than 60. The interaction of counterions with kraft lignin com-

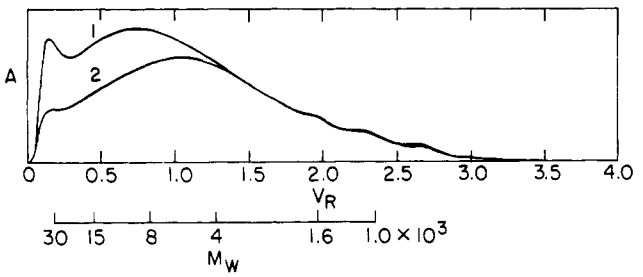



**Figure 7.** Effect of betaine on molecular weight distribution of kraft lignin: elution from Sephadex G75 with (1) 0.10 M aqueous NaOH and (2) 0.10 M aqueous NaOH containing 0.10 M betaine (monitored at 280 nm).

ponents is thus primarily a result of site binding since the effect appears to be favored by a small crystallographic radius for the cation. The bound counterions will be in equilibrium directly with those in open solution rather than with a local territorially bound population.[22]

Coordination of zwitterions to kraft lignin components would be expected to reduce the surface charge density of these species and change the effective $pK_a$'s of the anionic groups present. A pronounced reduction in the degree of association for the kraft lignin sample would be anticipated when the negative pole of the zwitterion possesses a lower intrinsic $pK_a$ than those of the phenoxide groups on the individual components. The possibility was investigated by eluting kraft lignin from Sephadex G75 with 0.10 M aqueous NaOH containing 0.10 M betaine. As shown in Figure 7, the proportion of higher molecular weight species approaching $\bar{M}_w = 30\,000$ was substantially reduced in the presence of the zwitterion. Since the absorptivity at 280 nm of the kraft lignin sample was found experimentally not to be affected by betaine, these observations indicate that the zwitterion does indeed promote dissociation between the lignin components in aqueous alkaline solution.

It is important to emphasize that the counterion and zwitterion effects illustrated here do not arise from polyion contraction of the kind observed, for example, with polysulfonates[23,24] or, more particularly, with lignin sulfonates.[25,26] The effects are preferentially directed toward the higher molecular weight kraft lignin species which are comprised primarily of associated complexes (Figure 4). Since these complexes appear to possess lamellar configurations,[5,27] their conformations should be largely unaffected by counterions or zwitterions held in their surrounding ionic atmospheres. This has been confirmed by a viscosimetric study of a paucidisperse kraft lignin fraction[17] consisting of high molecular weight associated complexes.[3] The intrinsic viscosity of the sample in aqueous solution was found to be independent of pH between 8.5 and ~13;[17] furthermore, in contrast with lignin sulfonates,[25,26] no increase in reduced viscosity with decreasing sample concentration could be detected.[17] Since the solute parameter governing size-exclusion chromatographic behavior is the hydrodynamic volume,[28] these viscosimetric results directly support the view that associated kraft lignin complexes are *conformationally* insensitive to counterions (and, in all likelihood, zwitterions) under the conditions employed in the present work.

As in all previous cases involving different degrees of association for the same kraft lignin sample, the ratios[12] of components in the subset with molecular weight below ~3500 remain constant; the counterion and zwitterion effects influence the relative proportions of only the higher molecular weight species (Figures 6 and 7).

**Fractional Precipitation of Kraft Lignin.** During the isolation of kraft lignin by acidification of black liquor, it would be expected that progressive protonation of the component anionic groups could, under appropriate conditions, allow the degree of association of the sample to increase as the pH is reduced. The compositions of precipitates consecutively isolated during the course of acidification might therefore reflect successive stages in the assembly of associated kraft lignin complexes.

**Table II**
**Yields of Kraft Lignin Fractionally Precipitated during Progressive Acidification of Black Liquor[a]**

| pH[b] | % kraft lignin[c] | pH[b] | % kraft lignin[c] |
|---|---|---|---|
| 9.5 | 0.5 | 6.5 | 40.8 |
| 8.5 | 0.4 | 5.5 | 45.1 |
| 7.5 | 8.2 | 4.0 | 5.0 |

[a] Using freshly diluted black liquor containing 170 g $L^{-1}$ dissolved solids; substantial differences in yields were found after extended incubation of stock solution prior to acidification. [b] Diluted black liquor initially at pH 12.1. [c] Taken together, the fractions represented 38% (w/w) of the total dissolved solids in black liquor.

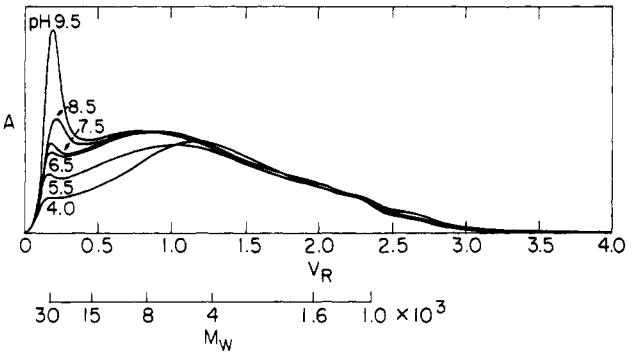



**Figure 8.** Fractional acid precipitation of kraft lignin from diluted black liquor during progressive acidification between pH 9.5 and 4.0 (Sephadex G75/0.10 M aqueous NaOH elution profiles monitored at 280 nm).

Such a possibility was investigated by progressively acidifying a batch of black liquor which had been freshly diluted so as to contain 170 g $L^{-1}$ dissolved solids (see Experimental Section). The solution (containing solid suspension) was consecutively centrifuged at 6 different pHs between 9.5 and 4.0 until separation of each precipitate was complete; no further precipitation below pH 4.0 was observed.

The yields of the kraft lignin fractions thus obtained from freshly diluted black liquor over a 120-h period are listed in Table II; the respective profiles described by elution through Sephadex G75 with 0.10 M aqueous NaOH are shown in Figure 8. It is evident that the relative proportions of higher molecular weight species decrease markedly with pH, while in contrast the components of the subset with molecular weight below ~3500 maintain constant ratios with respect to each other.

It is instructive to consider the possibility that, at any given pH, some fraction of the higher molecular weight kraft lignin species separates from solution to form particles which collectively constitute a precipitated phase. The remaining components could then be partitioned between the supernatant phase and occluded solvent water in this precipitated phase.[29] Partitioning into the precipitated phase would occur more favorably at lower pHs where the frequency of charged groups on the components would be smaller; the process would, however, exhibit a selectivity that increases exponentially with component molecular weight.[29] The implications of the proposal thus would not conform with the findings presented in Figure 8.

An alternative supposition would hold that the species which separate from solution are associated kraft lignin

complexes. As the pH is reduced, the decreasing charge density on the kraft lignin components would render associated complex formation more favorable. Complexes precipitating from solution at lower pHs would therefore embody larger proportions of lower molecular weight components. When these species are redissolved prior to elution from Sephadex G75 (Figure 8), dissociation will tend to establish characteristic proportions of complexes and components that remain relatively stable under the strongly basic conditions prevailing in 0.10 M aqueous NaOH. Providing that the components of the subset with molecular weight below ~3500 associate in constant ratios with respect to each other, this argument would account adequately for the observed molecular weight distributions of the kraft lignin fractions. That stoichiometric constraints should in effect act upon associative processes involving kraft lignin components demands careful examination.

**Mode of Association.** A particular aspect of the manner in which betaine influences the apparent molecular weight distribution of kraft lignin has revealed that the associative process involves at least two kinetically distinguishable steps. Preincubation of the kraft lignin sample in aqueous NaOH containing betaine, followed by elution from Sephadex G75 with 0.10 M aqueous NaOH alone, resulted in elution profiles identical with those observed when prior incubation was carried out under the same conditions but without the zwitterion. Thus a sudden reduction in the concentration of betaine allows a rate of reassociation which is at least 2 orders of magnitude faster in 0.10 M aqueous NaOH than that observed had the zwitterion not been initially present.[30] These findings can be rationalized in terms of a slow (rate-controlling) process leading to component conformations which are compatible with subsequently rapid associated complex formation:[4]

discrete components $\xrightleftharpoons{\text{slow}}$ conformationally compatible components $\xrightleftharpoons{\text{rapid}}$ associated complexes

The variation of weight-average molecular weight, $\bar{M}_w$, with number-average molecular weight, $\bar{M}_n$, for a lignin sample during the course of association can provide quite specific information about the mode of the process. The relationship between the two parameters is given by[4]

$$\partial\bar{M}_w/\partial\bar{M}_n = 2\langle m_i m_j\rangle(1/\bar{M}_n^2)$$

where $\langle m_i m_j\rangle$ at any particular point is the appropriate ensemble average product of the molecular weights of associating components. Expressions deduced for $\langle m_i m_j\rangle$ should be consistent with the preceding scheme; they can be evaluated numerically from the populations of lignin species empirically observed during the associative/dissociative process. Direct comparison with experiment is facilitated by considering a plot of $\bar{M}_w$ vs. $1/\bar{M}_n$, the slope of which at any point is equal to $-2\langle m_i m_j\rangle$.

The dependence of $\bar{M}_w$ on $1/\bar{M}_n$ that characterizes the dissociation of the gymnosperm kraft lignin sample in 0.10 M aqueous NaOH (Figure 4 and Table I) is illustrated in Figure 9. The plot indicates that $\langle m_i m_j\rangle$ remains effectively constant in magnitude ($2.22 \times 10^7 \pm 3.7\%$) while the formal degree of association[15] between the kraft lignin components varies from 0.45 to 0.

The constraints upon the molecular transformations underlying the associative/dissociative processes are most conveniently identified by considering the system explicitly in kinetic terms. Thus discrete components which cannot

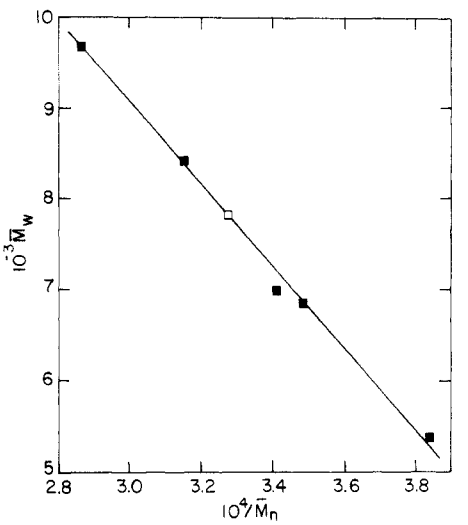



**Figure 9.** (■) Relationship between $\bar{M}_w$ and $\bar{M}_n$ during dissociation of kraft lignin components present at 1.0 and 0.5 g $L^{-1}$ in 0.10 M aqueous NaOH (Table I). (□) Effect of 0.10 M betaine on $\bar{M}_w$ and $\bar{M}_n$ of kraft lignin in 0.10 M aqueous NaOH.

participate directly in association are denoted by $L_{di}$; these species undergo a slow conformational change to components $L_{ci}$ that may then interact productively with complexes $C_j$. Inclusion of the respective rate coefficients allows the overall scheme to be summarized as follows:

$$L_{di} \underset{k_{-ci}}{\overset{k_{ci}}{\rightleftharpoons}} L_{ci} \underset{k_{-aij}}{\overset{k_{aij}[C_j]}{\rightleftharpoons}} L_iC_j$$

Assuming that a steady-state concentration is maintained for $L_{ci}$, the net rate of association, $v_j$, for complexes $C_j$ is given by

$$v_j = \sum_i \left\{ k_{aij}[C_j] \frac{(k_{ci}[L_i] + \sum_j k_{-aij}[L_iC_j])}{(k_{ci} + k_{-ci} + \sum_j k_{aij}[C_j])} - k_{-aij}[L_iC_j] \right\}$$

where $[L_i] = [L_{di}] + [L_{ci}]$. Note that for each term in the overall summation, the $C_j$'s represent those complexes possessing loci compatible with the respective components $L_i$. Since $\sum_j k_{aij}[C_j] \gg k_{ci} + k_{-ci}$ (vide supra), the expression for $v_j$ can be written

$$v_j = \sum_i \left\{ \frac{k_{aij}[C_j]}{\sum_j k_{aij}[C_j]} (k_{ci}[L_i] + \sum_j k_{-aij}[L_iC_j]) - k_{-aij}[L_iC_j] \right\}$$

If $\langle m_i m_j\rangle$ is to remain constant during association, the species whose *relative* concentrations vary (namely those with molecular weights greater than 3500) must exhibit zero-order kinetic behavior. There is one constraint alone that will meet this condition: each complex $C_j$ must associate *exclusively* with a particular component $L_i$ so that $k_{aij}$ is nonzero *only* for a given $i$ and $j$. Hereby the rates of association, $v_j$ and $v_i$, for the complementary species $C_j$ and $L_i$ become

$$v_j = v_i = k_{ci}[L_i]$$

When $k_{ci}$ is independent of $i$, the average molecular weight, $\langle m_i\rangle$, of associating components $L_i$ remains constant during the process and is given by

$$\langle m_i\rangle = \frac{\sum_i m_i v_i}{\sum_i v_i} = \bar{M}_{ni}$$

where $\bar{M}_{ni}$ is the number-average molecular weight of the

component subset $\{L_i\}$. The assumption that $k_{ci}$ should be independent of $i$ is quite reasonable if productive encounters occur when the *same* minimum number of (effectively independent) chain segments in each $L_i$ is conformationally compatible with part of the corresponding locus on $C_j$; subsequent conformational rearrangements can then lead to interactions between all potentially complementary segments on $L_i$ and $C_j$.

It has been consistently observed (vide supra) that the ratios of kraft lignin components with molecular weights below 3500 do not vary with the degree of association for the sample as a whole. $\bar{M}_{ni}$ may thus be correctly identified as the number-average molecular weight ($1.55 \times 10^3 \pm 3.6\%$) of this oligomeric component subset. The appropriate average molecular weight, $\langle m_j \rangle$, for the complementary species $C_j$ (with molecular weights greater than 3500) is calculated to be

$$\langle m_j \rangle = \frac{\sum_j m_j v_j}{\sum_j v_j} = 1.54 \times 10^4$$

when the concentrations of associating components $L_i$ are equal to one another. Significant variations with molecular weight of the absorptivity at the wavelength (280 nm) employed in monitoring Sephadex G75/0.10 M aqueous NaOH elution profiles prevent this condition from being confirmed directly for the oligomeric kraft lignin components. Indirect confirmation is, however, provided by the value for $\langle m_j \rangle$ obtained from

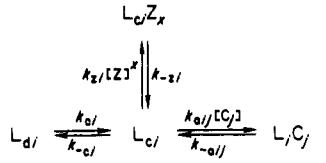
$$\langle m_j \rangle = \frac{\langle m_i m_j \rangle}{\langle m_i \rangle} = 1.43 \times 10^4 \pm 5\%$$

which lies within 7% of that deduced above from the apparent molecular weight distributions.

The available data therefore suggest that the mode of association between gymnosperm kraft lignin species is quite specific: not only do the higher molecular weight complexes interact selectively with lower molecular weight oligomers, but complexes of a given type are evidently compatible with only one kind of component. Simply stated, the associated complexes appear to be, in some sense, topologically regular so that the associative process is stoichiometrically constrained. Herein lies the justification for the straightforward method adopted in the present work for determining the formal degree of association for kraft lignin samples.[15]

That two kinetically distinguishable steps should govern the associative phenomenon was originally inferred from the effect of betaine upon the molecular weight distribution of kraft lignin in aqueous alkaline solution (vide supra): the zwitterion is assumed to perturb the faster of the two coupled processes by blocking the protonation of components $L_{ci}$ implicitly included in the phenomenological rate coefficient $k_{aij}$. Yet the molecular weight distribution of kraft lignin in 0.10 M aqueous NaOH containing 0.10 M betaine accurately conforms with the relationship between $\bar{M}_w$ and $\bar{M}_n$ that characterizes the slow dissociation of the same sample in aqueous alkaline solution alone (Figure 9).

In order to examine under what circumstances it is consistent with the proposed kinetic scheme, the influence of the zwitterion upon the associative equilibrium is most economically ascertained by considering its interaction with components that are conformationally disposed for productive encounters with complexes $C_j$: coordination of betaine to $L_{ci}$ will prevent these species from participating directly in the primary association step. (The zwitterion undoubtedly coordinates also to components $L_{di}$ which are conformationally incompatible with association, but this will not affect the outcome of the argument.) Thus betaine may be incorporated into the kinetic scheme as follows:



Here $k_{zi}$ is the phenomenological rate coefficient for the coordination of $x$ zwitterions Z with $L_{ci}$, and $k_{-zi}$ is that for their displacement from the sites on the component.

The net rate of association, $v_i$, between complementary species $L_i$ and $C_j$ now becomes

$$v_i = k_{aij}[C_j]\frac{(k_{ci}[L_i] + k_{-zi}[L_{ci}Z_x] + k_{-aij}[L_iC_j])}{k_{ci} + k_{-ci} + k_{zi}[Z]^x + k_{aij}[C_j]} - k_{-aij}[L_iC_j]$$

At equilibrium, since $v_i = 0$

$$\frac{k_{ci}[L_i]_{eq}}{k_{ci} + k_{-ci}} = \frac{k_{-zi}[L_{ci}Z_x]_{eq}}{k_{zi}[Z]^x} = \frac{k_{-aij}[L_iC_j]_{eq}}{k_{aij}[C_j]_{eq}}$$

In as far as $k_{ci}$ and $k_{-ci}$ are independent of $i$, $[L_{ci}Z_x]_{eq}$ will be directly proportional to $[L_i]_{eq}$ if $k_{-zi}/k_{zi}[Z]^x$ does not vary with $i$. This condition implies that for each component $L_{ci}$ the train of chain segments facilitating the initial productive encounter with $C_j$ should possess the same intrinsic affinity for the coordinated zwitterions. The concentration of $L_{ci}Z_x$ will, of course, remain very close to its equilibrium value when that of $L_i$ is far from equilibrium, providing that the rate at which the zwitterions interact reversibly with the components is very fast compared to interconversion between conformations $L_{ci}$ and $L_{di}$.

Reasonable assumptions, then, about the proposed kinetic scheme have permitted a working hypothesis to be formulated about the associative/dissociative processes that is in accord with the observed behavior of kraft lignin samples in aqueous alkaline solution. While their overall applicability is a subject of continuing investigations, one of these conditions has been of central importance for reducing the complete rate expression to a form consistent with experimental findings. It has hereby become apparent that association is not simply a random process: each kraft lignin complex possesses a locus which is complementary to only one type of component. Of course the chemical structures of the components that are compatible with a particular locus need not be identical in every detail, but they presumably have in common a dominant molecular feature which serves to distinguish between component types.

The contention that the associated complexes be topologically regular has been independently supported by the behavior encountered with subsets of such species isolable from complete kraft lignin samples. Using conventional regenerated cellulose tubing (with 24-Å average pore radius permeability), 72-h dialysis of Indulin AT against 0.10 M aqueous NaOH and then water[3] retains a kraft lignin fraction which, after precipitation and leaching with methanol, exhibits the apparent molecular weight distribution depicted in Figure 10 upon elution from Sephadex G75 with 0.10 M aqueous NaOH. The rate of dissociation observed for kraft lignin components in 0.10 M aqueous NaOH (Figure 4) indicates that the retentate from the

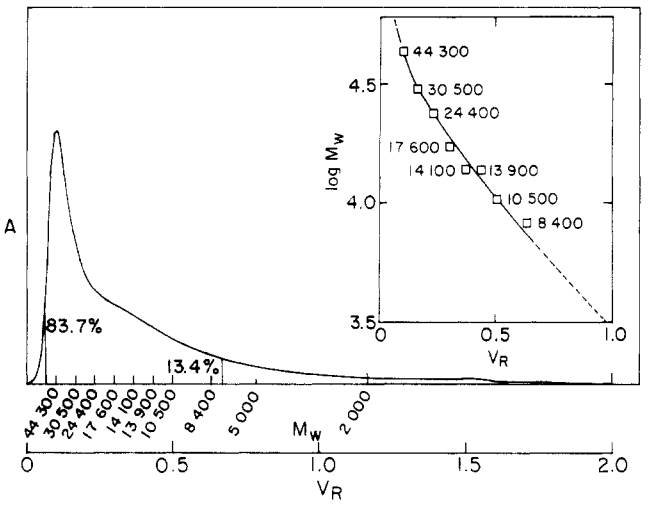



**Figure 10.** Molecular weight distribution of Indulin AT fraction remaining after dialysis for 72 h against 0.10 M aqueous NaOH, then water, followed by leaching of precipitated kraft lignin species with methanol[3] (Sephadex G75/0.10 M aqueous NaOH monitored at 280 nm). Inset: semilogarithmic plot of weight-average molecular weight vs. retention volume.

dialyzed sample consists primarily of associated complexes. The weight-average molecular weights for eight paucidisperse fractions selected from the elution profile were deduced by ultracentrifuge sedimentation equilibrium studies of solutions each made up to pH 9.5 so as to contain 2.5 $\times 10^{-2}$ g $L^{-1}$ kraft lignin species. The values similarly obtained at pH 8.5 were, within experimental error, identical. (The resulting calibration curve shown in the inset of Figure 10 is parallel to that determined for the Douglas fir kraft lignin using the same batch of Sephadex.) The weight-average molecular weight ($4.18 \times 10^4$) for the whole dialyzed Indulin fraction determined directly by sedimentation equilibrium under the same conditions at pH 9.5 was much larger than that ($2.52 \times 10^4$) calculated from the apparent molecular weight distribution (Figure 10). The difference was even greater at pH 8.5 due to a further increase in the weight-average molecular weight of the whole dialyzed fraction as the pH was reduced. These observations indicate that the associative processes occurring within the subset of kraft lignin complexes are largely prohibited in paucidisperse fractions of the constituent species under the same conditions. It thus appears that the relative proportions, rather than absolute concentrations, of the different complexes present in solution most significantly influence the extent to which association between these entities can occur.

The topological regularity inherent in associated kraft lignin complexes must be related to the structural features of the components from which they are assembled, but the overwhelming majority of these molecular species remain to be characterized. Exhaustive acetylation of lignin samples does not appreciably affect the relative proportions of high molecular weight complexes observed in nonaqueous solvents such as DMF,[31] so hydrogen bonding cannot provide the driving force for association. On the other hand, hydrophobic interactions alone would not be sufficient to overcome the electrostatic replusion between polyionic lignin components associating in aqueous alkaline solution. Presumably nonbonded orbital interactions between the aromatic moieties of the components govern the underlying mechanism of these associative processes. Semiempirical molecular orbital calculations[32] suggest that the structures of the complexes could be based upon a head-to-tail orientation between the substituted benzene rings of the interacting components

The apparent dimensions of monomolecular lignin films disposed on a water surface[33] have been taken to imply that the species present in such monolayers are "disklike" in shape.[27] Since the monolayers were spread from solutions in which association between lignin components is strongly favored,[3] it seems probable that associated lignin complexes possess lamellar configurations.[5] Discrete kraft lignin components have been found to behave like appreciably expanded random coil molecules in aqueous alkaline solution; no hydrodynamic effects arising from long-chain branching were observed, but the results from these investigations were insensitive to short-chain branching.[5] If the individual components associate to form a lamellar structure with aromatic moieties disposed perpendicularly to the plane of the species, short-chain branches extending from the surfaces could oppose aggregation of these topologically regular complexes into a lattice. Certainly crystalline domains have never been detected in any lignin preparation or derivative.[34] Perhaps these circumstances are roughly comparable to the difficulties encountered in attempts to crystallize hemicelluloses with frequent short-chain branches.[35]

The results from the present work have presented a view which conflicts with that generally accepted about the nature of kraft lignins. When a macromolecule constituted by a supposedly random proportionate distribution of eight different linkages between (*p*-hydroxyphenyl)propane units[36] is subjected to the relatively severe conditions encountered during kraft pulping (typically at 170 °C for 2 h in aqueous solution containing 45 g $L^{-1}$ NaOH and 12 g $L^{-1}$ $Na_2S$), an almost hopelessly complicated mixture of degraded and partially "condensed" products might be expected.[37] No fewer than 22 of the monomeric and 16 of the dimeric components from spruce kraft lignin have been fully characterized,[38] and on this basis alone it would not be unreasonable to assume that the higher molecular weight fractions should contain an enormous variety of species. However, since they were fractionated under conditions in nonaqueous solvents that favor association,[3] the monomers and dimers were isolated in relative yields[38] that do not reflect their actual proportions among the individual components taken as a whole. It is difficult to contemplate that associated kraft lignin complexes could be topologically regular unless the constituent components within each narrow molecular weight range were to possess a limited number of dominant structural features. Recent studies carried out at the University of Minnesota have indeed indicated that paucidisperse kraft lignin fractions may be predominantly composed of relatively few discrete molecular species.[39]

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**Registry No.** Kraft lignin, 8068-05-1.

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(13) The critical chain length, in general, is the minimum degree of polymerization necessary for participation in associated complex formation.[14] This chain length has been exceeded by the members of the lower molecular weight kraft lignin oligomeric subset since all of these components take part in the associative process.
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# Evidence for the Existence of Associated Lignin–Carbohydrate Polymers As Revealed by Carbon-13 CPMAS Solid-State NMR Spectroscopy

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ABSTRACT: $^{13}C$ CPMAS NMR spectroscopy was used to examine the relaxation profiles in solid samples of *Piceae glauca* wood pulp. Proton spin–lattice ($T_{1H}$) and proton rotating-frame spin–lattice ($T_{1\rho H}$) relaxation techniques were utilized in conjunction with induced paramagnetic relaxation, $^{13}C$–$^{1}H$ interrupted-proton decoupling, and enzymatic degradation to infer details of structure in the wood pulp constituents. Divergent $T_{1H}$'s in this material were seen to converge toward a singular value as nonbound or remotely bound cellulose units were degraded and removed from the solid-state sample matrix. This observation suggests that the residual lignocellulosic heteropolymer was associated in such a manner that proton spin diffusion was constant for all components, providing evidence for the existence of naturally occurring lignin–carbohydrate structures.

## Introduction

The nature of lignin–carbohydrate interactions in plant tissues has been a subject of increasing speculation in recent years. Mechanisms whereby lignin–carbohydrate bonds are formed have been proposed in synthetic model systems.[1-3] The understanding of both the characteristics and the stability of lignocellulosic linkages is an important scientific concern since lignocellulosic polymers are major constituents of vascular plants and are widely utilized organic materials. The lignin component of deciduous lignocellulosic plant cell wall matter is known to inhibit the enzymatic breakdown of bound cellulose.[4,5] The efficient disruption of these interactions is of prime importance in such diverse processes as paper pulping, biomass conversion, and in the digestibility of forage in ruminants.

Standard $^{13}C$ Fourier-transform solution NMR techniques have been demonstrated to be very useful for the examination of isolated lignin and cellulosic compounds, as well as in studies of soluble lignin–carbohydrate complexes and model organic compounds.[6-9] However, the isolation of soluble lignocellulosic compounds is somewhat problematic in that chemical treatment and destructive methods result in structural modification of the components present in these systems as evidenced in their $^{13}C$ NMR spectra.[9]

The advent of solid-state $^{13}C$ NMR employing cross polarization and magic angle spinning (CPMAS) has generally overcome many of the difficulties associated with reduced molecular motion in solids: $^{13}C$–$^{1}H$ dipolar coupling, chemical shift anisotropy and $^{13}C$ sensitivity prob-

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