MOLECULAR WEIGHT DISTRIBUTION OF ASPEN LIGNINS FROM CONVENTIONAL GEL PERMEATION CHROMATOGRAPHY, UNIVERSAL CALIBRATION AND SEDIMENTATION EQUILIBRIUM

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SUMMARY

Molecular weight distributions of ball-milled, organosolv, alkali-extracted/mild acid-hydrolyzed and alkali-extracted/steam-exploded aspen lignins in tetrahydrofuran were compared using conventional gel permeation chromatography (GPC), universal calibration and sedimentation equilibrium. Molecular weight averages reported in the literature from universal calibration for the four low-molecular-weight lignins agreed more closely with values found in this study from sedimentation equilibrium experiments than from conventional GPC. This result supports the suggestion that these acetylated lignins fit universal calibration.

INTRODUCTION

Lignins are complex, cross-linked phenylpropane polymers that compose, with cellulose and other carbohydrates, the cell wall structural members of living plants. They constitute 15–25% of the dry weight of material found in hardwoods[1–3]. Further progress in understanding the macromolecular properties of lignins now requires a reliable method for determining the molecular weights (MW) and molecular weight distributions (MWD) in a solvent that minimizes solute–solute, solute–solvent and solute–column interactions. Important contributions have been made using packed-bed and high-performance size-exclusion chromatography (HPSEC) in both neat and mixed organic solvents with underivatized[4–6] and methylated, silylated or acetylated lignins[7–13], and in aqueous sodium hydroxide with underivatized lignins[14–17], but a chromatographic system that performs optimally has not been reported. Tetrahydrofuran (THF), however, works well in minimizing solute–column and solute–solute interactions and, although limited in its ability to solubilize lignins over a wide range of MW, is suitable for use with polystyrene–divinylbenzene (*e.g.*, µStyragel) column packing materials[18]. Hydrophilic, high-dielectric solvents such as form-

amide[19], dimethyl sulfoxide (DMSO)[20] and dimethylformamide (DMF)[21] solubilize higher-MW underivatized lignins, yet perform poorly with μStyragel packing materials because of excessive bead instability[18]. Chromatography in DMF also results in extreme sensitivity to solute–column interaction.

Two commercial detectors introduced recently have greatly extended the capability of SEC, the real-time differential viscometer (DV) and the low-angle laser light-scattering (LALLS) photometer. On-line viscometric detectors allow the determination of unknown polymer MWs using the principle of universal calibration[22–26]. On-line LALLS detectors and software systems apply Debye theory as improved by Zimm and Stockmayer[27] to find MWs and the root mean square radii for unknown polymers. These methods allow the real-time calculation of "true" MWs for unknown polymers from SEC and have been reviewed extensively[28–31]. An important limitation of LALLS is that values of the change in refractive index with solute concentration (dn/dc) for the polymer in a pure solvent must be precisely known and problems arise when polymers are heterogeneous in composition with respect to MW, because dn/dc will then also vary with MW.

This paper reports the first direct comparison of results from MWD analyses using conventional GPC, universal calibration via HPSEC–DV and sedimentation equilibrium for four acetylated hardwood lignins in THF. The lignin samples were obtained from aspen (*Populus tremuloides*) wood meal by ball milling, steam explosion followed by alkaline extraction, organosolv pulping and dilute sulfuric acid hydrolysis followed by sodium hydroxide extraction.

EXPERIMENTAL

Chemicals and standards

All chemical and HPSEC eluents were obtained from J. T. Baker (Phillipsburgh, NJ, U.S.A.), Fisher Scientific (Pittsburgh, PA, U.S.A.) and Aldrich (Milwaukee, WI, U.S.A.). The MW standards used to calibrate the three column system were obtained from American Polymer Labs. (Mentor, OH, U.S.A.) [polybutadienes (narrow MWD), poly-α-methylstyrenes (narrow MWD), poly(methyl methacrylates) (broad MWD)] and Polymer Labs. (Shropshire, U.K.) [polystyrenes (narrow MWD) and poly(methyl methacrylates) (narrow MWD)]. Two synthetic polymers prepared by anion-initiated polymerizations of a quinone methide (QMa) according to the procedure of Chum *et al.*[32] were treated as intermediate-MW lignin models.

Lignin samples

Ball-milled (BM) aspen lignin was prepared following the procedure of Lundquist *et al.*[33]. Alkali-extracted/steam-exploded (AESE) aspen lignin samples were prepared from steam-exploded wood pulp as described by Chum *et al.*[12]. Alkali-extracted/acid hydrolysis (AH/NaOH) lignin samples were prepared by subjecting aspen wood flour to a 1-hour cook at 120°C in 0.025 M sulfuric acid[34] and mixing the clarified supernatant with 1% (w/w) NaOH at 25°C with a Waring blender. The organosolv (OS) lignin was prepared from the liquor obtained by extracting aspen wood flour with methanol–water (70:30, v/v)[35]. Lignin samples were acetylated following the method of Gierer and Lindeberg[36]. Lignin samples were stored frozen during the study.

Chromatographic system

The chromatographic system used to evaluate conventional GPC analysis consisted of a Hewlet-Packard Model 1090M liquid chromatograph equipped with a Hewlett-Packard Model 1037A high-sensitivity refractive index (RI) detector and a Hewlett-Packard Model 1040A diode-array detector using signals at 260 nm with a band width of 80 nm. The column system was composed of three 30 × 7.8 mm I.D. columns (Beckman μSpherogel of pore size 10 000, 1000 and 500 Å) connected in series in order of increasing pore size. Narrow MWD standards (see *Chemicals and standards*) were used to obtain a linear calibration graph from $1 \cdot 10^6$ to 500 daltons. Lignin samples were injected at a concentration of 1.0 mg/ml with an autoinjector setting of 225 μl. Experimental data were reduced to statistical molecular weight averages using the Hewlett-Packard Chromatography Software with GPC upgrade.

Partial specific volume determination

Densities and apparent partial specific volumes of lignin samples in THF were determined using a Mettler/Paar Model DMA60 six-place precision digital density meter equipped with a DMA602 measuring cell. During measurement the sample temperature was controlled to within ±0.005°C using a Cascade Systems thermal circulator. The experimental protocol and instrument calibration followed a procedure described by Elder[37]. Air-free, deionized water was prepared just prior to use following the procedure of Wagenbreth and Blanke[38]. Lignin samples were prepared in THF at a concentration of 3 mg/ml by weighing to ±0.0005 mg; samples at concentrations of 1.5 and 0.75 mg/ml were prepared by serial dilution. All samples were prepared and analyzed in duplicate. Five measurements were taken for each sample when the time-lapse measurement reached equilibrium. Weighted-average values for these measurements were used for the density calculations.

Analytical ultracentrifugation

Sedimentation equilibrium studies were performed on a Beckman Model E ultracentrifuge equipped with RTIC temperature control and electronic speed control. Acetylated lignin samples at a concentration of *ca.* 1.8 mg/ml were normally allowed to reach equilibrium at 15 000, 30 000 and 40 000 rpm at 20°C. Rayleigh interference fringe patterns were recorded on Kodak Spectroscopic IIG film and analyzed on a Nikon Model V12 microcomparator equipped with a Nikon digital *x–y* stage. To improve the quality of the interference fringes at higher rotor speeds, the instrument light source was fitted with a polarizing filter[39] and the cell was assembled with sapphire windows, custom-cut window gaskets of polyamide sheet stock (0.013 cm thickness) and window liners of polyaramide sheet stock (0.025 cm thickness). To maintain a leak-free cell for THF solutions, special center-piece gaskets were cut from 0.010-cm thick Kel-F (3M) sheet stock.

Although double-sector center-pieces molded from Kel-F (currently unavailable from Beckman) deformed at elevated rotor speeds in the presence of THF and were therefore unsuitable for extended sedimentation equilibrium runs, they could be scribed as necessary to form a synthetic boundary center-piece. The short-duration, low rotor speed of the synthetic boundary forming experiment, necessary to find the initial solute concentration, c_o, was acceptable for THF exposure.

The right-hand cell sector was filled with 0.02 ml of FC-43 fluorocarbon and

0.120 ml of sample and the left-hand sector was filled with 0.160 ml of HPLC-grade THF. Synthetic boundary experiments for c_o determinations used 0.120 ml of sample and 0.360 ml of THF in the right- and left-hand sectors, respectively.

Theory of universal calibration

The concept of universal calibration, as introduced by Benoit *et al.*[22], is based on the Einstein viscosity law:

$$[\eta] = vN\upsilon_h/M \tag{1}$$

This equation relates the hydrodynamic volume, υ_h, of a macromolecule of molecular weight M to its intrinsic viscosity, $[\eta]$, in cm^3/g; N is Avogadro's number and v is a shape factor developed by Simha[40]. Also, as $[\eta]$ can be expressed as a function of the form $[\eta] = AM^x$, the familiar relationship,

$$[\eta] = K'M^a \tag{2}$$

first expressed by Mark[41] and Houwink[42] in the 1940s, was found. Here K' and a are the Mark–Houwink constants and are specific to a polymer–solvent–temperature system[43].

The principal of universal calibration is based on eqn. 1, which predicts that all molecules having the same value of $[\eta]M$ would have the same value of υ_h. Also, if υ_h is the parameter that uniquely determines the elution volume, V_e, these molecules should have the same elution volume. Universal calibration, then, permits the calculation of M for polymers of unknown structure from column elution data, without a knowledge of the Mark–Houwink constants, using a set of calibrated columns for which a calibration graph of $[\eta]M$ versus V_e (found using well characterized polymer standards) is known. Sources of error in this approach arise from both experimental SEC limitations and theoretical considerations[44,45].

Theory of sedimentation equilibrium

The expressions that describe the equilibrium concentration of solutes in the ultracentrifuge cell have been derived from both classical thermodynamics and material transport theory[46]. Svedberg[47,48] derived expressions from both approaches and showed that identical results can be found from both methods for ideal solutions. An equation that was found suitable for application of Rayleigh interference optics to ultracentrifuge data[49], assuming solution ideality, is

$$M_{app} = \{2RT/[(1 - \bar{\upsilon}\rho)\omega^2]\} (d\ln c/dr^2) \tag{3}$$

where c is concentration in fringes, r is the radial distance from the center of rotation in cm, T is time, $\bar{\upsilon}$ is the partial specific volume, ρ is the density of the solution, ω is the angular velocity of the rotor and R is the gas constant.

For homogeneous systems, plots of $\ln c$ versus r^2 yield straight lines. In general, systems indicating polydispersity show upward-sloping curves of $\ln c$ versus r^2 and non-ideal systems show downward-sloping curves[50]. Also important in determining the MWD of polydisperse polymers, although seldom applied, is multiple rotor speed

analysis. This method, developed by Fujita[51] and improved by Osterhoudt and Williams[52] and Scholte[53], permits the description of the total MWD of a sample of high polydispersity from MW data collected at multiple rotor speeds for various heights in the sample fluid column. Unfortunately, this method has not been translated directly for use with data collected with the interference optical system reported in this study[54].

Calculation of sedimentation equilibrium results

The Raleigh interference fringe photographs are aligned in the microcomparator and analyzed by the methods described by Chervenka[55] and Richards and Schachman[50]. The total weight-average molecular weight is estimated by the "conservation of mass" method first described by Lansing and Kraemer[56] and later developed by Richards *et al.*[57] and others. Here, a specific and limited form of eqn. 3 is used:

$$M_{app} = \{2RT/[(1 - \bar{v}\rho)\omega^2]\}\,(c_b - c_m)/[c_o(r_b^2 - r_m^2)] \tag{4}$$

where c_b, c_m and c_o represent the concentration of solute in fringes at the upper fluid meniscus, at the cell bottom (sample fluid column bottom–FC-43 interface) and in the original sample before loading, respectively. Values given in r, radial distance in rotor coordinates (cm), define these points in the cell. Further, we have assumed that at the 1–2 mg/ml solute concentrations used in this study we can assume ideal behavior in THF and therefore the data presented were collected at one concentration only.

RESULTS AND DISCUSSION

Chromatographic methods

The aspen wood lignin samples chosen for this study were prepared by organosolv, steam explosion, dilute acid hydrolysis and ball-milling procedures. Fig. 1 and 2 show the elution profiles of these four lignin samples using refractive index (RI) and UV detection, respectively. In comparison, elution profiles obtained with UV detection indicated minor variances with the RI curves at early elution (*e.g.*, high MW) for all samples analyzed. These differences in the tail of the elution envelope, which probably reflect different dependences of absorptivity on molecular weight for the two detection methods, may result in the differences in M_z calculated from these data and shown in Table I.

Table I also illustrates the results of applying "conventional GPC" analysis to the four lignin samples. A sensitive UV detector permitted the chromatograms to be recorded at low loadings (0.22 mg per injection). The values of the number-average (M_n), weight-average (M_w) and z-average (M_z) molecular weight obtained proved to be consistent with those reported by other laboratories[7-10] where a μStyragel (or similar) gel system in THF was used. The general trend shown by these data was the similarity in M_w between all the acetylated aspen lignins tested except the ball-milled lignin, which had a higher M_w. The polydispersities probably ranged within experimental error, *i.e.*, 3.2 (RI detection) for ball-milled aspen lignin to 4.8 (UV detection) for the steam-exploded lignin. The synthetic quinone methide-derived polymers were considerably narrower in polydispersity (near 1.5).

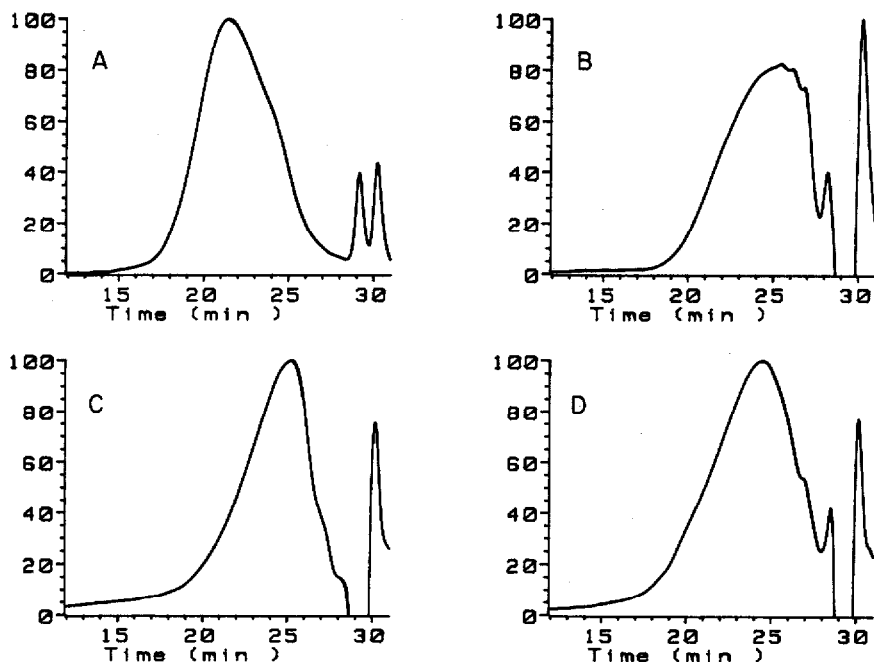We recently reported[58] the use of universal calibration to analyze the MWD of

Fig. 1. High-performance size-exclusion chromatography of four acetylated aspen lignin preparations: (A) ball-milled; (B) organosolv; (C) alkali-extracted/dilute acid-hydrolyzed; and (D) alkali-extracted/steam-exploded. Elution curves were obtained with a refractive index detector and displayed in normalized amplitude with the Hewlett-Packard Chromatography Software. Multiple positive and negative peaks near the total column elution volume (28–31 min) reflect elution of non-polymeric compounds and injection solvent and were not included in the MWD analysis. The sample loading was 0.22 mg per injection.

four acetylated aspen lignins and two quinone methide-derived polymers identical with those used here. Table II compares the molecular weight averages found for these polymers in that earlier study, using a Viscotek HPSEC–DV detector, with values found in this study by conventional GPC. The relative ordering of the values found by HPSEC–DV is very similar to those from conventional GPC (Table II), with ball-milled aspen lignin showing the highest molecular weight averages. However, the values of M_n from conventional GPC range from identical within experimental error (AESE) to values that are 35% smaller than those from universal calibration (OS and AH/NaOH), to values from GPC that are half of those from universal calibration (ball-milled and model polymers). The values of M_w are 20–40% smaller by conventional GPC for AESE, OS, AH/NaOH and ball-milled aspen lignin and are a factor of two smaller for the model polymers. There is a systematic difference in M_z of a factor of *ca.* 2 from the averages of conventional GPC and universal calibration, with the GPC values always being larger (lignins) and smaller (model polymers) by the same factor. This may indicate a detector- or software-based bias (in both GPC and HPSEC–DV data systems), as the placement of baselines on the original chromatographic data is especially critical in calculating the high-molecular-weight-sensitive M_z values. The polydispersities are the same within experimental
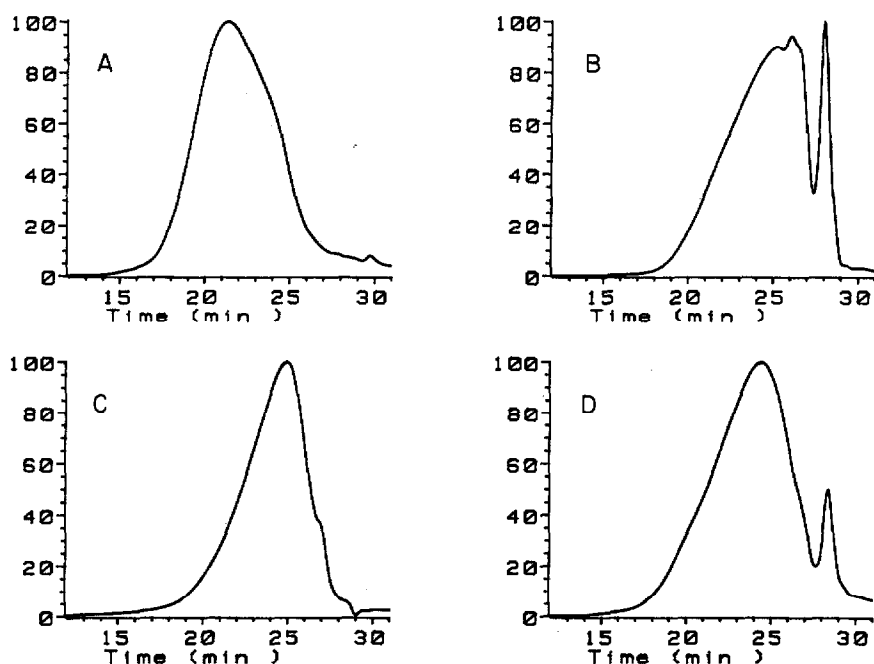
Fig. 2. High-performance size-exclusion chromatography of the acetylated lignins shown in Fig. 1. Elution curves were obtained with a UV detector at 260 nm and the sample loading was 0.22 mg per injection.

TABLE I

MWDs OF ACETYLATED ASPEN LIGNINS AND LIGNIN MODEL POLYMERS

From conventional GPC at different loadings and detection systems. GPC in THF at 20° with UV detection at 260 nm and a band width of 80 nm for low loadings, and 270 nm with a band width of 10 nm for the higher loadings. Low loadings were produced from solutions of 1.0 mg/ml and injections of 225 μl; high loadings were produced from solutions of 8.0 mg/ml with the same injection volume.

Sample[a]	Loading (mg)	M_n		M_w		M_z		M_w/M_n	
		UV	RI	UV	RI	UV	RI	UV	RI
AESE	0.22	1200	1300	5600	5100	88000	44000	4.7	3.9
AESE	1.7	1300	1400	6300	5900	110000	62000	4.8	4.2
OS	0.22	680	880	2800	2800	28000	9500	4.1	3.2
OS	1.7	610	840	2800	3400	64000	52000	4.6	4.0
AH/NaOH	0.22	1100	1100	4600	3700	120000	43000	4.2	3.4
AH/NaOH	1.7	1100	1200	4500	4300	95000	62000	4.1	3.6
BM	0.22	2800	3000	12000	12000	130000	120000	4.3	4.0
BM	1.7	3000	3100	10000	10000	58000	55000	3.3	3.2
QM 34	0.25	3710	--	5070	--	6320	--	1.4	--
QM 33	0.25	3930	--	7350	--	10170	--	1.9	--

[a] Acetylated lignins: alkali-extracted/steam-exploded, AESE; organosolv, OS; alkali-extracted/dilute acid-hydrolyzed, AH/NaOH; and ball milled, BM. QM indicates quinonemethide-derived model polymers.

TABLE II

MWDs OF ACETYLATED ASPEN LIGNINS AND MODEL COMPOUNDS: COMPARISON OF VALUES FROM UNIVERSAL CALIBRATION WITH AVERAGE VALUES FROM CONVENTIONAL GPC

Sample	Loading (mg)	M_n	M_w	M_z	M_v	M_w/M_n
AESE[a]	1	1100	7300	34 500	3300	6.7
AESE[a]	2	1900	7100	27 000	–	3.7
From GPC[b]	1.7	1400	5900	62 000	–	4.2
OS[a]	1	1300	5200	16 000	3200	4.0
OS[a]	2	1000	4400	18 000	–	4.4
From GPC[b]	1.7	840	3400	52 000	–	4.1
AH/NaOH[a]	1	2200	8100	38 000	4600	3.7
AH/NaOH[a]	2	1300	6600	34 000	–	5.0
From GPC[b]	1.7	1200	4300	62 000	–	3.6
BM[a]	1	3600	17300	46 800	11500	4.7
BM[a]	2	9000	22000	47 000	–	2.4
From GPC[b]	1.7	3100	10000	55 000	–	3.2
QM 34[a]	0.25	8700	10300	12 400	9900	1.1
From GPC[b]	0.25	3710	5070	6 320	–	1.4
QM 33[a]	0.25	14700	16700	20 000	16000	1.1
From GPC[b]	0.25	3930	7350	10 170	–	1.9

[a] Universal calibration values from Himmel *et al.*[58]. Obtained in THF at 20°C with RI detection and Unical 2.71 software (Viscotek). For high loadings, injections (250 µl) were made from 8 mg/ml stock solutions. For low loadings, injections (250 µl) were made from 4 mg/ml stock solutions.
[b] From RI detection values in Table I.

error for all lignin samples. In contrast, the polydispersities found for the quinone methide-derived polymers by universal calibration are near 1.1.

Sedimentation equilibrium

Values of the partial specific volume for the acetylated AESE, organosolv, AH/NaOH and ball-milled aspen lignins in THF found with the Mettler–Paar mechanical oscillator were 0.689, 0.665, 0.656 and 0.657 ml/g, respectively. Interestingly, these values are very similar to that of 0.663 ml/g found for unacetylated kraft lignin in dilute NaOH by McNaughton *et al.*[59]. These values were used to calculate a series of molecular weight averages for the lignin samples at various rotor speeds using the conservation of mass method (eqn. 5). Table III shows the results of these experiments at lignin concentrations of 1.8 mg/ml.

The issue of rotor speed variance and its impact on "intrinsic" M_w requires further discussion. Early work by Fujita[51] and Adams[60] clearly indicated the need to examine polydisperse samples at a wide range of ultracentrifuge rotor speeds. As the centrifugal force (proportional to ω^2) is increased on the cell and sample fluid column, the apparent distribution of components in the sample changes as heavier fractions sediment to the cell bottom (or lie close to this position). Work by Scholte[53] improved Fujita's early methods so that widely polydisperse polymers could be fully analyzed by extrapolation to zero rotor speed. Unfortunately, Scholte's work contributed precise methods for analysis of schlieren patterns only. Our work was per-

TABLE III

MWDs OF ACETYLATED ASPEN LIGNINS FROM PRELIMINARY SEDIMENTATION EQUI-
LIBRIUM IN THF AS A FUNCTION OF ROTOR SPEED

Lignin concentrations were 1.8 mg/ml. The values obtained at the lowest rotor speed are considered to be
the best approximation for M_w by sedimentation equilibrium and should be used for comparison with
values in Tables I and II.

Sample	Rotor speed (rpm)	$M_{w,app}$
AESE	15 000	9100
	30 000	5400
OS	15 000	8000
	30 000	3100
	40 000	2300
AH/NaOH	15 000	9000
	30 000	4900
BM	15 000	10600
	20 000	10200
	30 000	5200
	40 000	2400

formed with the Rayleigh interference optical system so that more dilute solutions
could be studied [schlieren optical analysis requires solute concentrations in the range
3–6 mg/ml]. Such extrapolations are not yet possible with our present data. We can,
however, infer from Scholte's work that values found for apparent M_w at low rotor
speeds (*i.e.*, 15 000 rpm) lie near to, and slightly lower than, the intrinsic M_w for the
sample. In this paper we shall consider these values for $M_{w,app}$ obtained at 15 000 rpm
to be a good approximation of M_w. Future work must focus on the development of
the theory necessary to perform the extrapolation of interference optical data to both
zero solute concentration and zero rotor speed.

CONCLUSIONS

Reconciliation of internal data

The approach outlined here is certainly not new in philosophy, yet we have
employed instrumentation and methodologies both very old and very new to this
problem. Historically, the task of bringing together multiple disciplines to the prob-
lem of polymer MWD analysis has been slow. Early work (1948) by Wales[61] and
Wales *et al.*[62] attempted to reconcile results from intrinsic viscosity, sedimentation
equilibrium and osmometric experiments for polydisperse materials (polystyrenes) in
organic solvents. In 1970, Sokolov *et al.*[63] performed little known but landmark work
in which approach-to-equilibrium methods were used to describe lignin in a variety of
solvents. Also important were the contributions of Pla and Robert[64–66], who gath-
ered hydrodynamic data from viscometry, sedimentation velocity and GPC on spruce
lignins in THF. They showed that spruce lignins exhibit typical behavior from plots
of log M versus log [η] indicative of branched polymers. Kraft lignin MWDs in
sodium hydroxide have also been extensively studied more recently using short-col-
umn sedimentation equilibrium by Sarkanen and co-workers[15,17]. Such examples of
multidisciplinary examination of lignin MDWs are limited, however.

One important aspect of this study was that all lignin samples, sample handling, column calibration standards, mobile phase (THF) and chromatographic column sets used for all experiments (including those from ref. 58) were the same. Hence significantly greater control of experimental variables resulting from laboratory-specific differences in these conditions and instrumentation was achieved. This consideration renders the differences shown in Tables I and II meaningful.

Although evidence exists that concentration effects may be important with even acetylated lignins in THF, increasing the column loadings from 0.25 to 1 mg seems unlikely to be the cause of the differences shown in Table II. These data identify poor sensitivity as a more general and pressing problem in state-of-the-art, SEC-based "absolute" MW analysis. From our work, the limiting value for sample loading appears to be near 0.1 mg per injection for conventional GPC (RI detector) and 1 mg per injection for HPSEC–DV. Loadings for HPSEC–LALLS are commonly reported[31] to be between 0.2 and 1 mg per injection. An increase in sensitivity of one order of magnitude would be highly valuable and should be an area of focus for suppliers of SEC detection equipment. For comparison, the non-SEC-based method, sedimentation equilibrium equipped with Rayleigh interference optics or UV scanning, has a detection limit of *ca.* 1 or 0.1 mg/ml, respectively. The SEC elution of polymers (lignins) at concentrations many orders of magnitude below this can, of course, be achieved by UV detection, but calculations are then limited to only conventional GPC methods.

In this study we compared the results from three hydrodynamic approaches to unknown polymer MW determination. Conventional GPC analysis from polystyrene-calibrated columns produced the lowest MW estimates for the four lignins and quinone-methide-derived polymers. Universal calibration methodology led to higher MW estimates (1.5–2.5 fold) than conventional GPC, and sedimentation equilibrium produced values for apparent MW that were similar to those from universal calibration. This result is not surprising, because conventional GPC examines the elution of a calibration series ordered on the basis of effective hydrodynamic volume, not MW. A similar result was recently found by Wooten *et al.*[67] in a study of the M_w found for "resole" phenolic resins in 0.1 M NaOH by conventional GPC and sedimentation equilibrium, where the M_w from the latter was five times greater than that from GPC. Universal calibration, however, relies on the conservation of the relationship between the hydrodynamic volume ($[\eta]M$) and elution volume for a specific column set throughout a wide range of polymer chemistries and sizes. Indeed, finding higher apparent MWs from universal calibration than from GPC is consistent with the concept of lignin being a branched polymer (*e.g.*, a branched polymer of higher MW may occupy the same hydrodynamic volume as a linear polymer). The ultimate conclusion of this study is that the low-MW, acetylated aspen lignins examined appear to fit universal calibration; however, comparison with data from LALLS (if possible) and from a refinement of sedimentation equilibrium analysis performed at multiple rotor speeds should eventually confirm or disprove this conclusion.

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