Characterization of Methocel™: Correlation of Static Light-Scattering Data to GPC Molar Mass Data Based on Pullulan Standards

DREW S. POCHÉ,[1] AL J. RIBES,[2] DEBBIE L. TIPTON[2]

[1] Department of Chemistry and Physics, Southeastern Louisiana University, Hammond, Louisiana 70402

[2] The Dow Chemical Company, Analytical Sciences, Plaquemine, Louisiana 70767

Received 10 November 1997; accepted 20 April 1998

ABSTRACT: Using low-angle laser light-scattering (LALLS) and multiangle laser light-scattering (MALLS) techniques, absolute molar mass averages were determined for a wide variety of polymers in the Methocel™ product line produced by The Dow Chemical Company. These data were correlated to GPC molar mass averages obtained from column calibration using pullulan standards. It was determined that the pullulan equivalent molar mass averages overestimated the values determined by light scattering by a factor as high as 3.2. Mark-Houwink parameters were calculated based upon values for the pullulan standards and application of the universal calibration concept. Sample preparation issues and chromatographic conditions that impact data quality are also presented. © 1998 John Wiley & Sons, Inc. J Appl Polym Sci 70: 2197–2210, 1998

Key words: aqueous GPC-LALLS; cellulose ethers; water-soluble polymer characterization; Methocel™ characterization; pullulan characterization; polysaccharide characterization

INTRODUCTION

High-performance gel permeation chromatography (HP-GPC) analysis of water-soluble polymers has been the subject of many articles over the last few decades.[1,2] A commonly reported problem involves competing nonsize exclusion separation mechanisms due to selective adsorption (or other binary or ternary interactions involving the mobile phase, sample, and column packing) of the polymer on the column packing material. This phenomenon is particularly troublesome in the analysis of charged polymers, such as certain proteins or polyelectrolytes. In addition, a lack of suitable water-soluble standards continues to burden conventional GPC analysis of certain water-soluble polymers. Problems such as these often compromise the integrity of the molar mass averages obtained from aqueous GPC analysis. Recognizing the potential problems in aqueous GPC analysis mentioned above, Omorodion, Hamielec, and Brash[3] reported a systematic analysis of nonionic polyacrylamides in an effort to optimize peak resolution and band broadening parameters.

The column-packing shortcomings have improved with the development of robust column packing materials. Original column packings for aqueous GPC work were commonly chemically modified, porous glass beads[4] or lightly crosslinked dextran or agarose.[5] Although the crosslinked polymers improved the sample adsorption characteristics over glass beads, their "softness" or nonrigidity rendered them unsuitable for the high flow rates and pressures associated with modern, high-performance chromato-

Correspondence to: D. S. Poché.

Journal of Applied Polymer Science, Vol. 70, 2197–2210 (1998)
 CCC 0021-8995/98/112197-14

graphic characterization. In the 1980s, manufacturers[6] began producing more rigid particles based upon densely crosslinked, hydroxylated, poly(methylmethacrylate). The hydrophillic nature of the surface combined with high mechanical strength have alleviated many of the historical problems with aqueous column-packing materials, provided a mobile phase of suitable ionic strength is employed.

For the characterization of cellulose or its derivatives, GPC standards are still a nagging concern. Usually poly(ethylene oxide) (PEO), dextrans, or pullulans are used to calibrate a column set. Both PEO and pullulan standards have been used by us for Methocel characterization. Calibration standards must have an identical hydrodynamic size compared to the sample under the same analysis conditions or the analysis only yields an "equivalent" molar mass, which may be widely different from the real molar mass of the sample. The column set used for this study was calibrated with narrow molar mass distribution pullulan standards. It is known[7] that pullulan, though a polysaccharide, is much more flexible due to the α-1,6 linkages of D-glucose and, hence, has more conformational freedom than a cellulose ether where repeat units are bonded only by β-1,4 linkages. In fact, cellulose ethers (e.g., hydroxypropyl cellulose) are commonly referred to as "semiflexible,"[8] acknowledging the rigidity in the chain. Under the same GPC mobile phase conditions, the hydrodynamic size of pullulan for a given molar mass is significantly smaller than the hydrodynamic size of a Methocel sample of identical molar mass. As such, the GPC calibration curve of log(M) versus retention time based upon pullulan standards overestimates the molar mass for the Methocel products in question. Other workers[9–13] have also noted this discrepancy when analyzing chitin or chitsosan derivatives (both, like Methocel, having the β-1,4-ether linkage of the repeat units).

There have been, of course, other methods used to compute molar mass averages. For example, Striegel and Timpa[14] recently used conventional polystyrene standards for column calibration (and nonaqueous GPC columns) using *N,N*-dimethylacetamide/lithium chloride[15] as the solvent and mobile phase. They analyzed cellulose fibers as well as pullulans and dextrans using the universal calibration method.[16] The shortcoming of this method is the necessity of acurately knowing the Mark-Houwink parameters of each sample in the solvent used as the mobile phase. Fishman et al.[17] and Kato et al.[18] calibrated aqueous GPC columns based upon the radius of gyration (R_g) size parameter. Provided one knows R_g for the standards (either independently measured or manufacturer supplied), plotting R_g versus retention time may provide a useful, if not "universal," calibration. To extract the molar mass of an unknown, however, requires *a priori* knowledge of the M_w–R_g scaling relationship for the unknown. A simple method to obtain absolute molar mass averages for unknowns using readily available standards involves "correcting" the calibration curve using absolute molar mass averages for the unknowns obtained by light-scattering methods. Of course, this method is really only convenient and useful if the sample set to analyze stays constant (which is normally the case for production plant support work). As molar mass detectors based upon light scattering or viscometry detection become increasingly common, the lack of suitable standards becomes less awkward. However, in our experience, these detectors require a good deal of expertise to employ and are not generally commonplace in production labs unless an experienced analyst oversees their implementation and operation.

Nilsson et al.[19] and Jumel et al[20] have recently reported data on GPC coupled to light scattering analysis of Methocel™ and other water-soluble polymers. As one would expect, they found light-scattering detection suitable for the determination of reproducible, reliable molar mass averages. Nilsson et al. also reported that intrinsic viscosity measurements alone were inadequate for characterizing and comparing the polymers in his sample set. Huber,[21] using GPC-LALLS and intrinsic viscosity measurements combined with the universal calibration approach, calculated hydrodynamic size relationships and Mark-Houwink (M-H) parameters for a variety of linear and branched polysaccharides.

As reported here, we reduced the absolute molar mass analysis of the Methocel product line to a conventional, GPC characterization using only a concentration sensitive detector (differential refractive index). To accomplish this, the absolute molar mass averages for the Methocel samples were measured by both low-angle (LALLS) and multiangle laser light-scattering (MALLS) techniques, and these data compared to the averages obtained from a pullulan calibration curve to yield a correction equation. Other concerns were to determine if the columns chosen for the analysis interfered in any way with the molar mass

average determination (either by total exclusion, shearing of high molar mass fractions, or adsorption) and the requirements for reproducible sample preparation (e.g., to avoid sample aggregation).

Static Light-Scattering Background

Static light-scattering measurements provide the molecular size parameters of polymers dissolved in a suitable solvent. The scattered intensity, evaluated as the Rayleigh ratio, $\mathcal{R}_\theta$, is the intensity ratio of the scattered light from the polymer, and the scattered light from the solution and is proportional to M_w of the scattering species. When is measured as a function of both concentration and angle, eq. (1) is used to evaluate the data

$$\frac{Kc}{\mathcal{R}_\theta} = M_w^{-1}\left(1 + q^2\frac{\langle R_g^2\rangle_z}{3}\right) + 2A_2c \quad (1)$$

$$\text{in the limit of } \theta = 0,\ \frac{Kc}{\mathcal{R}_\theta} = \frac{1}{M_w} + 2A_2c \quad (2)$$

$$\text{in the limit of } c = 0,\ \frac{Kc}{\mathcal{R}_\theta} = \frac{1}{M_w} + \frac{q^2 < R_g^2 >_z}{3} \quad (3)$$

where K is an optical constant for the specific solution and is given by

$$\left(\frac{2\pi^2 n_0^2}{\lambda_o^2 N_A}\right)\left(\frac{dn}{dc}\right)^2,$$

N_A is Avogadro's number, n_o is the solution refractive index, dn/dc is the specific refractive index increment, q is the magnitude of the scattering vector ($q = 4(n_o\sin(\theta/2)/\lambda_o)$, θ is the scattering angle, λ_o is the *in vacuo* wavelength, c is the concentration, $\langle R_g^2\rangle_z$ is the z-average of the mean squared radius of gyration size parameter. Equation (1) is applied for MALLS experiments. Plotting $(Kc)/(\mathcal{R}_\theta)$ versus $\sin^2(\theta/2) + kc$, where k is a scaling constant, is known as the Zimm analysis,[22] and was used to obtain M_w, A_2, and $\langle R_g^2\rangle_z$ for some of the polymers in this study.

Restricting the scattering intensity measurements to one low scattering angle reduces eq. (1) to eq. (4), applied in LALLS measurements. Equation (5) represents the relationship when the effluent from GPC columns is monitored by the LALLS detector.

$$\frac{Kc}{\mathcal{R}_\theta} = \frac{1}{M_w} + 2A_2c \quad (4)$$

$$\frac{Kc_i}{\mathcal{R}_{\theta,i}} = \frac{1}{M_{w,i}} + 2A_2c_i \quad (5)$$

$$M_n = \frac{\Sigma c_i}{\Sigma\frac{c_i}{M_i}} \quad (6)$$

$$M_w = \frac{\Sigma c_i M_i}{\Sigma c_i} \quad (7)$$

$$M_z = \frac{\Sigma c_i M_i^2}{\Sigma c_i M_i} \quad (8)$$

The increment, i, in eqs. 5–8 represents the ith fraction eluting from the column. The value of c_i is evaluated from the DRI detector response. The value of $\mathcal{R}_{\theta,i}$ and ultimately $M_{w,i}$, come from the LALLS detector response. By assuming monodisperse fractions, $M_{w,i} = M_i$, making possible the calculation of the other molar mass moments from the GPC-LALLS data using eqs. (6)–(8). As it is written, eq. (5) also assumes constant dn/dc (see the Evaluation of dn/dc Values section) and A_2 values across the molar mass distribution of the sample. The A_2 term, which quantifies two-body interactions (e.g., excluded volume effects) in the solution, corrects for the concentration dependence of the scattering. At very dilute concentrations, typical of those in the GPC effluent, the A_2 term can be ignored. Reed,[23] in a comprehensive examination of errors in multidetector GPC analysis, evaluated the magnitude of the error that results when ignoring or underestimating A_2. The underestimation of M_w that occurs was small for diluted fractions in a GPC analysis, except in cases where A_2 is exceptionally large (e.g., polyelectrolytes dispersed in solvents of low ionic strength[23]). The M_w value for the stand-alone analysis method was determined, then, simply by integrating the LALLS response. The area under the response is proportional to the intensity of scattered light from the sample with known injection mass. In stand-alone LALLS measurements M_w was determined without reference to the DRI detector response.

EXPERIMENTAL

Conventional GPC, GPC-LALLS, stand-alone LALLS, and MALLS were used to analyze the

Table I Typical Methyl/Hydroxy Propyl Substitution for the Methocel™ Products in this Study

Product	% Methyl	% Hydroxypropyl
311	26.60	28.26
E75M	30.92	10.40
F50	28.39	5.85
K4MS	22.00	8.00
K15M	22.80	10.70
J5MS	17.92	24.53
J12M	18.80	24.70
J75MS	18.99	23.93
J75M(856)	18.80	26.10
856N	19.50	24.40

following Methocel products: E75M, F50, K4MS, K15M, J5MS, J12M, J75MS, J75M(856), and 856N. The number and letter designations correspond to differences in the chemical treatment of the cellulose feed stock. They reflect whether the feed stock was surface treated, and the content of methyl and hydroxypropyl side-chain substitution present. Table I shows the percentage of methyl and hydroxypropyl substitution for each product. In this report, when a "family" of products are referred to, the first letter in the product code will be stated as such: J-chemistry, to refer to J5MS, J12M, J75MS, J75M(856) as a group.

Sample Preparation

Aliquots of each product type were weighed into 4-oz glass bottles. The polymers were then dispersed by adding hot (nearly boiling) aqueous 0.01 *M* NaCl and mechanically agitating during cooling to room temperature. The samples were usually stirred or shaken overnight. As the LALLS response is very sensitive to scattering from dust particles, on-line filters placed before the LALLS detector reduced the amount of extraneous scattering and provided a quieter baseline.

dn/dc Measurements

A KMX-16 differential refractometer (ThermoSeparations Products, Inc.), operating at $\lambda = 632.8$ nm and 30.0 ± 0.01°C, was used to measure the specific refractive index increment, d*n*/d*c*. The KMX-16 was calibrated according to the vendor's recommended procedure using aqueous NaCl solutions in the concentration range of $c = 0.5$–2.0 g/100 mL. By measuring against 0.01 *M* NaCl, the value of $\Delta n/c$ was determined for solutions of the polymer dissolved in 0.01 *M* NaCl for a concentration range of 1–5 mg/mL. These data were extrapolated to $c = 0$ in a plot of $\Delta n/c$ versus c to provide the specific refractive index increment. Even though a diluted NaCl solution was used as the solvent, the polymer solutions were not dialyzed against the solvent during the measurements, which some researchers practice.[24] For sufficiently dilute binary systems, where one component is low concentration, the equilibration using dialysis is unnecessary.[24]

In another method, *dn*/*dc* was estimated based upon DRI detector response. The DRI detector was calibrated by injecting a pullulan standard of known mass and *dn*/*dc* and computing the area under the response. Then Methocel samples of unknown *dn*/*dc* but known concentration were injected. By computing the area of the detector response, the *dn*/*dc* could be estimated.

GPC-LALLS and GPC-DRI Measurements

The hardware consisted of a Hewlett Packard HP 1090 pump with a 100-vial autosampler together with a KMX-6 low-angle laser light-scattering photometer (ThermoSeparations Products, Inc.) followed by a Hewlett Packard HP 1037A Refractive Index detector. The chromatographic columns were two 7.8×300 mm mixed-bed TSK gel GMPWxl from TosoHaas, configured in series. These columns consist of densely crosslinked, hydroxylated PMMA. The exclusion limit, based on PEO, is 8×10^6 daltons. Theoretical plates[25] for the column set were calculated regularly and the columns replaced if the value fell below 25,000 plates/meter. The columns were calibrated with narrow pullulan standards (Polymer Labs, Inc.) in the molar mass range of 580–1.6×10^6 daltons. In the course of preliminary experiments, the tubing from the low pressure pump to the injection rotor valve was replaced by PEEK tubing (0.012″), and the stainless steel tubing from the injection valve to the column was replaced with PEEK tubing of 0.010′ internal diameter. Also, to decrease the shedding of corrosion from stainless steel tubing remaining, the chromatograph was passivated prior to the experiments. These steps were deemed necessary to reduce excessive background scattering in the LALLS response. To further decrease extraneous scatter, two on-line, low dead-volume filter holders from ThermoSeparation Products were used to support the on-line filters. A Millipore mixed esters of cellulose (cellu-

lose nitrate and acetate) filter (VMWP type) with a nominal pore size of 0.05 μm was placed after the low-pressure pump; and either a hydrophilic polyvinylidene fluoride 0.22-μm filter from Millipore (GVWP 013) or a mixed esters of cellulose 0.45-μm filter from Millipore (HATF type) was placed before the LALLS unit.

An interdetector delay (flow time between the LALLS and DRI detectors) of 6 s was calculated by running a narrow molecular weight Pullulan standard (380 K) in 0.01 *M* NaCl. Polymer Laboratories, Inc. Caliber GPC/SEC software v. 6.0 was used for the data acquisition and analysis.

The mobile phase was 0.01 *M* NaCl flowing at a rate of 1.00 mL/min. The water used for preparing the mobile phase, and all sample solutions was purified by an organic bed scrubber and a deionizer unit. It was then filtered by a Millipore Milli-Q PF plus water purification system using capillary-fiber ultrafiltration with a nominal 5,000 dalton molar mass cutoff.

The samples were injected at 50 and 100 μL volumes using concentrations in the 0.1–1.7 mg/mL range (depending upon sample molar mass). The analysis for each sample was usually performed in triplicate; analysis for some samples was repeated five or six times. The column oven was heated to 30°C. Beam attenuators 2, 3, and 4 were employed when setting the incident voltage on the KMX-6, P_0, to 500 or 640 mV. The annulus was set at 6–7°, and the field stop was 0.15 mm. The value used for the refractive index of the mobile phase was 1.333.

MALLS Measurements

The traditional Zimm analysis method[22] for multiangle static light-scattering data was used to compute M_w, A_2 and $\langle R_g \rangle_z^{1/2}$for a limited number of Methocel products. The MALLS instrument used in this study was an Otsuka DLS 700 model (vended by Polymer Labs, Inc.) equipped with a He-Ne laser operating at λ = 632.8 nm. The 21-mm (diameter) sample cell rested in a dibutyl phthalate refractive index matching bath during the measurements. The instrument was calibrated with toluene using the known Rayleigh factor for toluene at 90° scattering angle and incident light of 632.8 nm. The scattering intensity was measured at a sampling rate of 160 ms and 3000 data points were collected and averaged. The scattering from each concentration was measured from λ = 30–150° in 10° increments.

Five to seven concentrations were prepared for each product analyzed by the MALLS technique. The sample volume required for the measurement was 4 mL. Because the sample cell of the instrument is not designed as a flow-through cell, dust or particulate matter must be scrupulously removed from the samples. In "static" sample cells like this one, dust is not "pushed" out of the measured volume by a flowing mobile phase as in a GPC detector. Each freshly prepared solution was filtered through a 0.22-μm Anotop syringe filter prior to analysis. The solvent used to prepare the solutions was prefiltered through 0.02 μm Anotop syringe filters after addition of the NaCl. Furthermore, samples were checked for suspended particulate by visual inspection through the view port on the MALLS unit. The scattering intensity of the samples were monitored at 30° (lowest scattering angle used in the analysis) over several minutes. Dust appears as "spikes" over the time interval of the measurement. The vendor software allows the user to reject points 10% above the average. The software also allows the analyst to reject off-trend data in the reduced data set.

Stand-Alone LALLS Measurements

To allow for polymer dispersion into the mobile phase prior to the LALLS sample cell, about 30 feet of 0.010″ diameter tubing were used in place of the GPC columns. The concentrations injected were comparable to those used in the GPC analysis. The incident voltage, P_0 was 200 mV, attenuators used were 2, 3, and 4, and a 10-s signal filter was applied. The weight average molar mass, M_w, was determined from the known injection mass and total area under the LALLS response.

RESULTS AND DISCUSSION

Prefiltering Samples

We assumed initially that preparation of stock solutions at 0.5% w/v, stored refrigerated and diluted as needed, would adequately serve our sample preparation needs. We quickly discovered major discrepancies in the light-scattering data that turned out to be a function of the prefiltration step using syringe filters. Although the stored stock solutions were diluted by as much as 20-fold prior to filtration and analysis, the polymers were apparently highly aggregated even after dilution.

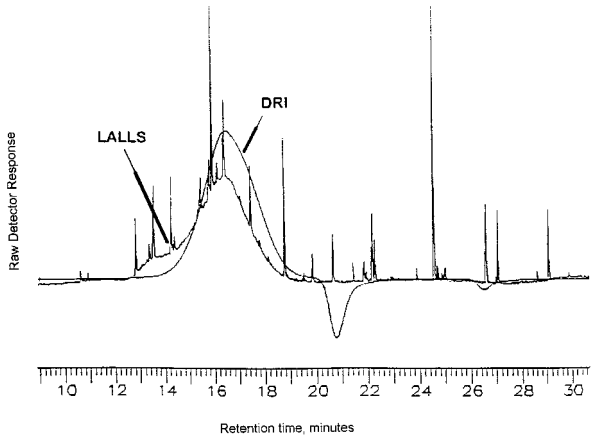

Figure 1 LALLS and DRI response for product F50 without prewetting.

Neely[26–28] showed that methyl cellulose forms aggregates (multimers), which increase as a function of temperature, time, methyl substitution, and solution ionic strength. Increasing the ionic strength of the solvent makes the aggregation problem worse according to Neely[26] (presumably because the polymer chain must "compete" with the ions for water solvation). However, a modest increase in ionic strength of the GPC mobile phase was deemed necessary to prevent the polymers from adsorbing to the column packing,[6] even though they were not charged. Interestingly, Neely also observed that lowering the temperature would break apart aggregates that had formed in aged solutions. We did not make this observation for the 0.5% w/v samples we refrigerated. Kuhn and coworkers[29] also observed aggregation phenomena for methyl cellulose. Their data suggested that lower molar mass polymers showed a greater tendency to aggregate.

The Methocel™ products in this study, while forming molecularly dissolved aqueous solutions, cannot be stored long term (even days) without aggregation. Colorimetric analysis[30] for carbohydrate content before and after filtering revealed that much of the polymer was being retained on the syringe filter. Though the severity of the apparent aggregation varied with the type of Methocel product, all of the products characterized displayed this behavior. Preparing the samples fresh and at the desired (much diluted) concentration directly circumvented the aggregation problem. As described in the Experimental section, the samples must be wetted prior to dissolution by dispersing the polymer in hot water, followed by cooling of the solution. Failure to follow the sample preparation in this manner led to abnormally high molar mass averages or poorly reproducible data. Shown in Figure 1 are early GPC-LALLS detector traces for product F50. Note the high molar mass "shoulder" and the "spiking" in the LALLS response. The "spiking" is due to large particulate (e.g., aggregates or dust) in the mobile phase. These anomalies disappeared when the sample was prepared as described above. After proper sample dissolution, all of the Methocel solutions described here could be prefiltered for the measurements using either a 0.20 or 0.45-μm syringe filter as appropriate without deleterious effects on the molar mass calculations. Table II shows the close agreement in the molar mass averages for F50 measured using 0.22 μm, 0.45 μm, and no syringe filters. For each of these runs, a 0.45-μm on-line filter was in place prior to the LALLS sample cell. This result for F50 is particularly satisfying because this product contains the highest ratio of methyl to hydroxypropyl substitution (Table I). The higher content of methyl side chains exacerbates the aggregation in aqueous solutions[26,31,32] (refs. 31 and 32 refer to hydroxypropyl substitution).

On-Line Filters

To perform light-scattering measurements at low angles, it was important to make the solution as dust free as possible. Scattering from dust will obscure scattering from the polymer. In good solvents, where there is little or no aggregation, the solutions can be cleaned with filters having porosities only slightly larger than the polymer. In poorer solvents the choice of filters can be harder. The porosity of the filter should not exclude any polymer but still remove most of the dust. Both 0.22 and 0.45-μm on-line filters, placed directly before the LALLS sample cell, were employed in this study. There was no appreciable difference in the average molecular weights obtained with ei-

Table II Stand-Alone LALLS Data for Product F50 Showing M_w Reproducibility with Various Prefiltering Procedures

Prefilter	M_z	M_w	M_n	M_w/M_n
0.45 μm	165 (14)	96 (6)	43 (5)	2.24 (0.18)
0.20 μm	170 (9)	95 (8)	42 (8)	2.30 (0.28)
None	153 (20)	91 (13)	39 (15)	2.39 (0.57)

Molar mass averages are in kilodaltons. Values in parenthesis are 2σ. All runs made with a 0.45 μm filter on line.

ther filter using GPC-LALLS, as shown in Table III. Ratios of M_w (0.45 μm)/M_w (0.22 μm) for a given product were nearly 1. This was important for two reasons: (1) the 0.22-μm filter should remove more dust than the 0.45-μm filter and, (2) as there was no evidence for aggregation of the polymer, the data were obtained under good solvent conditions and corresponded to single molecules. Indeed, the data in Table III indicate that on-line filtering with the 0.22 μm filter reduced scattering background in the LALLS response, making for a quieter baseline, as was demonstrated by the better reproducibility in the M_n and M_z moments. Regardless of filter pore size, the measurements all suffer from band broadening inherent in the chromatographic system. Also, some of the variation in the molar mass moments was due to the finite resolution characteristics of the columns and to mixing effects that occurred in the detector cells. These have no effect on the M_w, but will cause M_n to be higher and M_z to be lower than their true values[33] in a GPC-LALLS analysis. Finally, the M_n and M_z averages will always be more sensitive to baseline selection than the M_w moment.

Evaluation of *dn/dc* Values

An accurate knowledge of the specific refractive index increment, *dn*/*dc*, for the polymer/solvent combination is critical for meaningful absolute size parameters from static light-scattering measurements. As shown in the Background section, the value for *dn*/*dc* is squared in the calculation of M_w. Errors in M_w, then, increase with the reciprocal square of the *dn*/*dc* value.[23]

Given the disastrous effects inaccurate *dn*/*dc* values have on LALLS data, we considered the potential for variation of *dn*/*dc* with respect to the MMD. The two factors responsible for the variation are changes in molar mass and chemical composition of the repeat units. The *dn*/*dc* change due to a molar mass difference is really an issue of chain composition. Chain ends, which can be compositionally different particularly in synthetic polymers, become more prevalent on a number basis at lower molar mass. In naturally occurring polysaccharides (or their derivatives), chain ends are normally not substantially different from the repeat units in the chain, and *dn*/*dc* values for linear chains are constant over a wide molar mass range.

Variation in the composition of the repeat units is the issue we address here. Methocel products

Table III GPC-LALLS Data Showing the Agreement Using Either a 0.45-μm or 0.20-μm Filter On Line Before the LALLS Cell

	0.45 μm				0.22 μm				Ratio
Product	M_z	M_wA	M_n	M_w/M_n	M_z	M_wB	M_n	M_w/M_n	A/B
311	1,244 (236)	854 (111)	391 (70)	2.20 (0.51)	1,230 (25)	820 (25)	330 (36)	2.49 (0.20)	1.05
E75M	1,144 (68)	902 (45)	508 (66)	1.78 (0.16)	1,217 (61)	872 (17)	389 (39)	2.25 (0.22)	1.03
F50	167 (10)	96 (6)	43 (5)	2.23 (0.11)	170 (10)	95 (8)	42 (8)	2.30 (0.28)	1.00
K4MS	755 (38)	397 (32)	132 (17)	3.01 (0.21)	744 (30)	379 (11)	115 (5)	3.28 (0.10)	1.05
K15M	1,106 (110)	498 (25)	100 (58)	5.87 (0.76)	1,095 (55)	514 (21)	111 (19)	4.64 (0.70)	0.97
J5MS	999 (60)	487 (49)	106 (30)	4.65 (1.02)	876 (35)	449 (13)	113 (17)	3.98 (0.44)	1.08
J12M	1,126 (192)	658 (72)	201 (18)	3.28 (0.07)	1,179 (71)	657 (27)	180 (5)	3.65 (0.22)	1.00
J75MS	1,498 (194)	1,101 (121)	477 (5)	231 (0.25)	1,648 (16)	1,129 (34)	399 (52)	283 (0.25)	0.97
J75M (856)	1,544 (92)	1,025 (82)	346 (86)	3.00 (0.66)	1,733 (17)	1,108 (22)	313 (16)	3.54 (0.14)	0.91
856N	1,873 (168)	1,247 (50)	397 (67)	3.16 (0.60)	2,049 (348)	1,303 (104)	350 (108)	3.80 (1.37)	0.96

Molar mass averages are in kilodaltons. Values in parenthesis are 2σ. The data variation is smaller with the 0.20-μm filter on line.

may be considered terpolymers containing methyl substituted, hydroxypropyl substituted, and possibly nonsubstituted D-glucose repeat units. Because the substitution may vary across the molar mass distribution, so, in principle, may the *dn/dc* value. In general, this is a common problem when copolymers are analyzed by GPC light-scattering techniques.[34] Researchers have modeled or measured distribution of the substituents along the cellulose chain,[35–38] with no attention given to the influence of feed stock polydispersity on the substitution chemistry (although Gelman[38] mentioned this possibility). Certainly, how the cellulose feed stock is processed and the substitution chemistry carried out will influence the substitution across the MMD. Reaction conditions that break up the cellulose crystalline structure allow for more complete, random, chain substitution and, importantly, result in cellulose ethers that are highly soluble with no insoluble material.[39] The products in our study dissolved in water with virtually no insoluble fraction. Examples in the literature point to little or no difference in *dn/dc* values, with varying degrees of substitution (DS) for some polysaccharides. For example,[40] no change in *dn/dc* was noted for hydroxyethyl starch dissolved in 0.9% NaCl, with DS ranging from 0.06–1.2 (dn/dc = 0.151, 20°C, λ = 436 nm). Indeed, values for starch substituted with acetyl, hydroxybutyl , and hydroxypropyl side chains, in water, were identical to the value for hydroxyethyl starch.[40] Our *dn/dc* values measured for the "bulk" samples with the KMX-16 differential refractometer were in the narrow range of 0.128–0.132 mL/g, with an uncertainty of 4% despite substitution differences ranging from 5.85–28.26% hydroxypropyl ether side chains (Table I). The global average was determined to be 0.129 ± 0.004 (2σ).

Like polysaccharides, Methocel products contain a large number of free hydroxyl groups, both on the polymer backbone and in the hydroxypropyl side chains. Their interaction with water is responsible for solvating the polymer chain. Notwithstanding the effects due to different chain shapes (e.g., branched polysaccharides), to a large extent the high content of hydroxyl groups controls the magnitude of the refractive index of the solution.[41] As such, the *dn/dc* values measured here are in reasonable agreement with the values for other linear polysaccharides of different chemical composition in aqueous solution after accounting for the influence of λ_o on the values. For example, for hydroxypropyl cellulose (HPC) in water, a value of 0.131 mL/g (λ_o = 632.8 nm) has been reported[42] and for pullulan in 0.05 *M* NaCl, 0.135 mL/g (λ_o = 546 nm).[21] Only when a substantial amount of hydroxyl groups are "capped" with alkyl or ester sustituents (or when branching is present) do the values appear to change substantially (e.g., for methyl cellulose in water, dn/dc = 0.141 mL/g, value obtained by extrapolation to λ_o = 632.8 nm;[26,43] for acetyl starch DS =1.0, dn/dc = 0.141 mL/g, λ_o = 436 nm;[40] for dextran in water, dn/dc = 0.151 mL/g, λ_o = 436 nm[21]). Barth[41] measured the differential refractive index detector response of several different polysaccharides and noted that the detector response for a given concentration showed little variation between samples. In short, we assumed in this study that *dn/dc* is constant across a given sample's molar mass distribution and the preceding discussion tentatively supports this assumption.

The values were also determined for several products by the method of DRI detector calibration, and were in good agreement with the above-described values (average value of samples measured, 0.132 ± 0.014, 2σ). Equations (9) and (10) show the relationship of *dn/dc* to DRI detector response

$$k_{\mathrm{DRI}} = \left(\frac{c_{\mathrm{std}}}{A_{\mathrm{std}}}\right)(dn/dc)_{\mathrm{std}} \tag{9}$$

$$(dn/dc)_{\mathrm{unk}} = \left(\frac{A_{\mathrm{unk}}}{c_{\mathrm{unk}}}\right)k_{\mathrm{DRI}} \tag{10}$$

where k_{DRI} is the calibration constant for the DRI detector, A and c are the area under the detector response and concentration for the unknown (unk) and standard (std), respectively. The value of $(dn/dc)_{\mathrm{std}}$ used should correspond to the λ_o used in the light-scattering measurements. Although we used a polymer standard for calibration, Reed[44] suggested an aqueous NaCl solution for calibration. If the *dn/dc* value is known for the sample, then the DRI response can be used to calculate c [eq. (10)]. This, as Reed points out, is useful for determining whether the polymer sample is adsorbing on the column. Using eq. (10), the value of c_{unk} calculated from the *dn/dc* value should agree with the gravimetrically prepared value unless loss occurs on the column set. We did not observe significant loss of the Methocel sample on the columns using this method except when the samples were not prepared as described in the Experimental section.

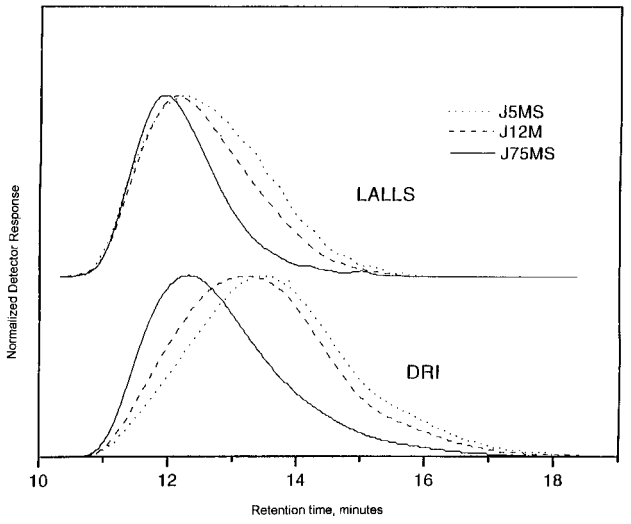

Figure 2 Typical GPC-LALLS data for J-chemistry products.

Evaluating Column Performance

Other concerns with the GPC analysis of Methocel included the possibility of anomalies in molar mass averages due to columns excluding the high molecular weight components and column shearing effects. To evaluate shearing effects, the M_w value obtained from GPC-LALLS experiments were compared with values obtained from the stand-alone LALLS experiments (no columns). For all but product F50, we obtained similar results from both experiments. The LALLS signal from F50 contained a very noisy baseline, and peak isolation was subjective, causing some uncertainty for the molecular weight of this product.

We noticed no exclusion of high molar mass fractions in any of the samples analyzed in this study. There was no evidence in the LALLS response of a sudden, steep, signal at the high end of the molar mass distribution, indicative of column exclusion. The narrow molar mass distribution pullulan standard with a nominal $M_w = 1.6 \times 10^6$ daltons eluted without exclusion. This corresponds roughly to a Methocel™ molar mass of about 5×10^6 daltons (see Correcting GPC Molar Mass Averages section). None of the samples in this study had M_z averages (from GPC-LALLS) exceeding 2.5×10^6 daltons.

Figure 2 shows typical DRI and LALLS detector responses for the J-chemistry products. After the sample aggregation phenomenon discussed above was addressed, most runs were free of spiking, and the quality of data shown in Figure 2 was typical. It is worth noting here that in the early stages of the study, we evaluated column temperatures above 30°C and found that the spiking in the LALLS response increased significantly. This supported the observation of methyl cellulose aggregation with increasing temperature reported by Neely.[26]

A comparison of the polydispersity ratio, M_w/M_n, in Tables IV and V show discrepancies between the ratios calculated from conventional GPC and the GPC-LALLS method. The polydispersity ratio from the two methods tend to differ for samples with broad molar mass distributions. Without band broadening corrections, GPC calculations overestimate the polydispersity ratio. GPC-LALLS measurements tend to underestimate the polydispersity ratio (the LALLS response gives M_w for the eluting fractions; due to

Table IV Pullulan Equivalent Molar Mass Averages for Methocel™ Products

Product	M_z	M_w	M_n	M_w/M_n
311	4,353 (233)	1,969 (208)	514 (301)	4.08 (2.01)
E75M	4,577 (46)	2,326 (138)	649 (50)	3.60 (0.53)
F50	929 (287)	278 (22)	82 (11)	3.41 (0.27)
K4MS	3,572 (65)	1,268 (4)	256 (53)	4.97 (1.10)
K15M	4,357 (37)	1,535 (31)	208 (24)	7.40 (0.72)
J5MS	4,234 (86)	1,546 (7)	194 (16)	7.97 (0.70)
J12M	4,311 (178)	1,753 (41)	281 (17)	6.23 (0.42)
J75MS	5,086 (81)	2,594 (189)	511 (68)	5.10 (0.73)
J75M(856)	5,098 (395)	2,496 (384)	390 (71)	6.46 (1.80)
856N	5,354 (362)	2,687 (391)	440 (132)	6.18 (1.54)

Molar mass averages are in kilodaltons. Values in parenthesis are 2σ.

Table V Pullulan Equivalent M_w Corrected with Eq. (13)

Product	Stand alone LALLS M_wA	GPC-DRI M_wB	B/A	Global Correction	Global % Error	Product Correction	Product % Error
311	909 (106)	1,969	2.17	762	16	844	7
E75M	891 (80)	2,326	2.61	968	−9	864	3
K4MS	395 (46)	1,268	3.21	404	−2	345	6
K15M	508 (58)	1,535	3.02	532	−5	529	−2
J5MS	502 (48)	1,546	3.08	538	−7	497	1
J12M	705 (56)	1,753	2.49	644	9	670	4
J75MS	1,010 (50)	2,594	2.57	1,133	−12	1,068	−6
J75M(856)	1,128 (122)	2,496	2.21	1,072	5	1,054	8
856N	1,216 (146)	2,687	2.21	1,192	2	1,219	0

Molar mass averages are in kilodaltons. % Error calculated assuming stand alone LALLS is the true value. Values in parenthesis are 2σ.

finite resolution of the column set, the $M_i = M_w$ assumption causes M_n to be overestimated when eq. (6) is employed). The two values bracket the true polydispersity ratio.[33]

Evaluating the Use of Pullulan Standards

In this section we compare the molecular weights obtained from GPC measurements using pullulan standards with those obtained using stand-alone LALLS measurements. Table IV shows typical pullulan equivalent molar mass averages for the Methocel products. Comparison of data in Tables IV, V, and VI clearly show the large differences in molar mass determined from the two methods. Using pullulan standards to calibrate columns will tend to overestimate M_w by as much as a factor of 3.2 (Table V). In an effort to improve the simple, traditional GPC technique, we used the static light-scattering data to "correct" the pullulan molar mass averages so that a more realistic estimate of molar mass could be obtained.

Table VI Mark-Houwink K and a Parameters Calculated Based upon $[\eta] = 0.036\ M^{0.635}$ for Pullulan

Product	$K \times 10^4$ mL g^{-1}	**a**
311	0.00458	1.56
E75M	0.0181	1.48
K4MS	4.17	1.15
K15M	6.93	1.23
J5MS	1.51	1.19
J12M	0.613	1.07
J75MS	0.0833	1.34
J75M(856)	0.838	1.17
856N	1.76	1.11

The error estimates for K and **a** are nominally 10 and 5%, respectively.

Correcting GPC Molar Mass Averages

The calibration plot obtained using pullulan standards can be converted to a "global" calibration plot for Methocel™ products by comparing the log of the molar mass distributions of the Methocel products obtained from GPC-LALLS as a function of retention time and the calibration line for the pullulan standards. Because the LALLS detector provides M_w of each fraction from the column, analysis of a polydisperse Methocel™ product by GPC-LALLS essentially calibrates the columns over a wide molar mass range for that particular product when $\log(M_w)$ is plotted against retention time. Examples of the $\log(M_w)$ versus retention time relationship for the pullulan standard set and several Methocel™ products, all fitted using a linear least squares regression, are shown in Figure 3.

Assume at some point t that the line obtained when plotting log M versus retention time for Methocel™ and the line from the same plot for pullulan standards intersect.

$$\text{GPC}-\text{LALLS: } \log(M_1) = b_1 + m_1 t$$

$$\text{GPC (pullulan): } \log(M_2) = b_2 + m_2 t$$

Then at retention time t,

$$\frac{\log(M_1) - b_1}{m_1} = \frac{\log(M_2) - b_2}{m_2} \qquad (11)$$

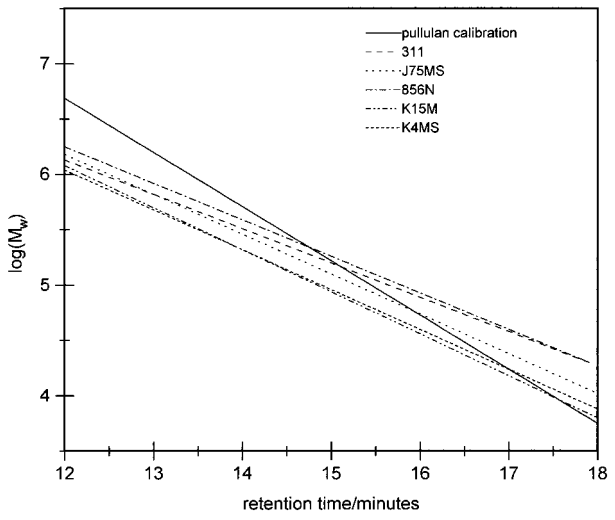

Figure 3 Column calibration curves. LogM_w versus rentention time for pullulan standards and several broad molar mass distribution Methocel™ products.

$$M_1 = 10^{\left(b_1 - \frac{m_1 b_2}{m_2}\right)} M_2^{\frac{m_1}{m_2}} \text{ or } M_{\text{corrected}} = aM_{\text{GPC}}^b \quad (12)$$

Using the slope and y-intercept data from plots like those illustrated in Figure 3, eq. (12) was used to provide a correction equation for $M_{w,\text{GPC}}$ based upon each product type. The corrections and errors for the M_w moment based on product type are shown in Table V.

A global correction equation for the M_w average was obtained from the plot of log$M_{w,\text{LALLS}}$ versus log$M_{w,\text{GPC}}$ (Fig. 4), and is shown in eq. (13). The term "global" refers to a correction applied to all Methocel™ products in this study. In eq. (13), a and b may be dependent upon chromatographic conditions (mobile phase, temperature, column packing). Once eq. (13) is determined, however, the relationship is useful because frequent changing of the established chromatographic method is unlikely. The M_w average in eq. (13) comes from the stand-alone LALLS data. The data in Figure 4 were fit with a linear least-squares regression. The correlation coefficient for the fit in Figure 4 is 0.9739. In principle, similar plots using the M_n and M_z averages could be constructed.

$$M_{w,\text{corrected}} = 6.47 \pm 1.67 \times 10^{-4} M_{w,\text{GPC}}^{1.44 \pm 0.13} \quad (13)$$

Molar mass data for F50, the product with the highest methyl/hydroxypropyl substitution ratio, were not used to derive eq. (13). The error estimate on a and b [eq. (12)] are represented by the standard deviation resulting from the linear least-squares regression. As the % error estimates in Table V show, the corrected M_w average based on the eq. (13) gave a reasonable approximation to the stand-alone LALLS M_w. Of course, the product corrections in general gave better approximations. The "noisy" linear fit reflected in the correlation coefficient (Fig. 4) may suggest the variation in hydrodynamic sizes of the polymer chain due to differences in the side-chain substitution ratios of methyl/hydroxypropyl (Table I). However, compared to the rather large error bars shown in the plot (represented as 2σ), this effect on the data was minor. For such a wide variety of Methocel™ products, these M_w corrections represented a significant improvement to pullulan equivalent averages.

The Mark-Houwink (M-H) parameters for the Methocel™ products may be estimated using the data illustrated in Figure 3 (logM_w vs. retention time) and knowledge of the Mark-Houwink parameters for pullulan. Assuming the validity of the universal calibration concept[17] for polymers with a shape factor[45] like our sample set, the M-H parameters for Methocel™ samples were determined as follows:

$$[\eta]_{\text{std}} M_{\text{std}} = K_{\text{std}} M_{\text{std}}^{a+1} \text{ and } [\eta]_{\text{unk}} M_{\text{unk}} = K_{\text{unk}} M_{\text{unk}}^{a+1}$$

at retention time t,

$$[\eta]_{\text{std}} M_{std} = [\eta]_{\text{unk}} M_{\text{unk}} \text{ or}$$

$$[\eta]_{\text{std}} M_{\text{std}} = K_{\text{unk}} M_{\text{unk}}^{a+1} \quad (14)$$

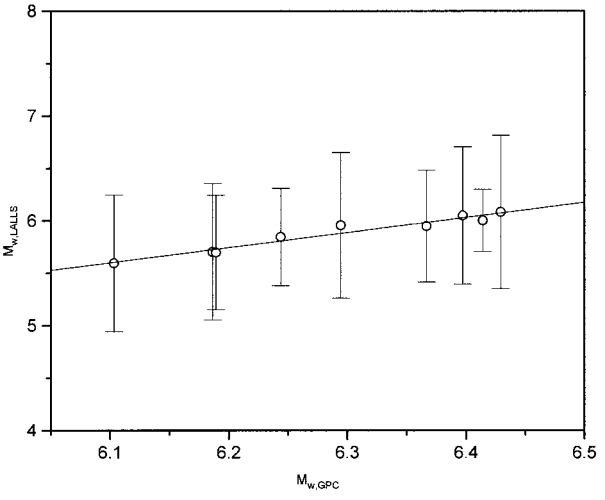

Figure 4 LogM_{LALLS} versus logM_{GPC} correction curve. The fitted data provide molar mass correction.

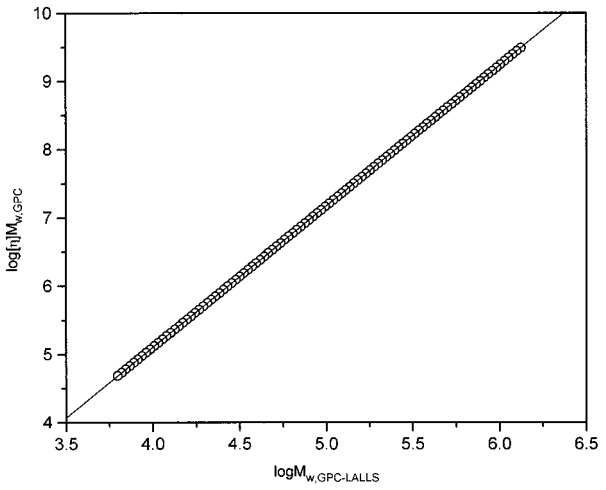

Figure 5 Plot of hydrodynamic volume versus GPC-LALLS M_w for J12M.

A similar analysis was published recently by Mrkvicková[46] in the analysis of graft copolymers. Because the product $[\eta]_{std}M_{std}$ can be determined from the M-H parameters for pullulan and M_{unk} was determined for each slice of the molar mass distribution of a product by GPC-LALLS (Fig. 3), K_{unk} and **a** for the product (unknown) may be determined from a plot of $[\eta]_{std}M_{std}$ versus M_{unk}. The M-H parameters used for pullulan obtained in solvent conditions similar to the study here (in 0.05 *M* NaCl) were **a** = 0.635 ± 5% and *K* = 0.0360 ± 10% mL/g.[21] An example plot of eq. (14) for product J12M is shown in Figure 5. There were 100 data points generated in the elution time range of 12–18 min. The M-H equation determined for J12M polymer was $[\eta] = 6.13 \times 10^{-5} M_w^{1.07}$. The M-H parameters determined by this method for the other products are compiled in Table VI. Consistent with the observed column elution behavior of the products compared to pullulan, the **a** values for the Methocel™ products were considerably larger than that of pullulan. A couple of **a** values in Table VI appear overestimated (e.g., products 311 and E75M), because values in the range of 1.3–1.5 compare with **a** values reported for polymers known to be rod-like.[47] However, it is interesting to note that, more or less, the values for **a** cluster around 1.2 (average of **a** values in Table VI is 1.25 ± 0.34, 2σ), despite differences in substitution chemistry (Table I), which should, to a large degree, control the compactness of the polymer chain in solution. Other workers have reported **a** values in water at 25°C for methyl cellulose from **a** = 0.55[26] to **a** = 1.0,[40] depending upon the degree of substitution of methyl groups (increased methyl substitution gives lower **a** values). The polymers of this study, of course, differ from methyl cellulose due to large amounts of hydroxypropyl substitution. Wirick and Waldman[48] reported **a** = 1.17 and later[40] reported **a** = 0.917 for HPC in ethanol at 25°C . In fact, the rigidity of the HPC chain is sufficient to cause formation of a lyotropic liquid crystalline phase in aqueous solutions and several workers have studied this behavior.[42,49]

MALLS Data

Table VII shows the results obtained for MALLS measurements on four Methocel products. The M_w values generally compared favorably to the stand-alone LALLS and GPC-LALLS values, again supporting the assumption that little or no shearing occurred with the column set used in this study. Oddly, M_w for product K4MS was significantly higher than the LALLS value. As filtering prior to measurement was crucial for clean samples using the "static" sample cell of the Otsuka instrument, the data in Table VII also suggest that the syringe filters had no deleterious effect on the samples.

The values of A_2 and $\langle R_g \rangle_z^{1/2}$ are also reported in Table VII. The negative values of A_2 for F50 and J5MS may imply (though does not prove conclusively) mild aggregation in these samples. Except for sample 311, the $\langle R_g \rangle_z^{1/2}$ values hint at an extended chain conformation in 0.01 *N* NaCl. In light of the negative A_2 values (and sample polydispersity in general), the R_g to M_w relationship should be interpreted cautiously. Because light-scattering measurements give the *z*-average of the square of R_g, the technique weights the bigger polymers more heavily in the average. This weighting is severe in polydisperse samples, and will be even more pronounced if aggregates are present.

Table VII MALLS Data for Selected Samples

Product	MALLS M_w	A_2/mol-mL/g^{-2}	R_g/nm
F50	113	−0.0069	33.9
K4MS	567	0.0182	70.2
J5MS	430	−0.0014	68.2
311	1,008	0.0044	78.3

Molar mass averages are in kilodaltons.

CONCLUSION

GPC, GPC-LALLS, stand-alone LALLS, and MALLS have been performed on several Methocel products. The molecular weight average obtained by GPC using pullulan as standards was shown to be overestimated by as much as a factor of 3.2. A correction factor for the GPC results was presented, allowing a simple GPC method using pullulan calibration standards to be developed for routine analysis of Methocel production plant products. Using M-H values for the pullulan standards and applying the concept of universal column calibration, M-H values for the products were calculated. Methods for reproducible sample preparation and column evaluation were presented. Samples must be prepared fresh and used immediately for meaningful GPC analysis. The present column set was adequate for analyzing the products currently produced by the Methocel production plants without compromising the integrity of the molar mass averages obtained from the characterization. Future work on the solution characterization of these products must include viscosity measurements, which would help provide a better understanding of the hydrodynamic properties and would remove the inherent uncertainty in the M-H values reported here.

Future characterization work must include measurements with a viscometry detection for more reliable and accurate determinations of the M-H parameters. Concerns about the variation of *dn/dc* with the MMD of a sample may be addressed by fractionating samples and characterizing the fractions and/or by evaluating the DRI detector response across the distribution to determine if the response is linear with concentration.

The authors wish to thank Mr. Steve Gregory (Midland, MI) and Ms. Sue Dallessaudro (Midland, MI) for managerial support of this project. We also wish to acknowledge the technical expertise of Dr. Nitis Sarkar (Midland, MI), Mr. Sergio Cutie (Midland, MI), Dr. David Meunier (Midland, MI), Dr. Michael Smith (Plaquemine, LA, for colorimetric analysis), and Mr. Rob Harrell (Plaquemine, LA, for *dn/dc* measurements).

REFERENCES

1. M. Potschka and P. L. Dubin, Eds., *ACS Symp. Series,* **635,** 13, 17, 19 (1996).
2. P. L. Dubin, Ed., *Aqueous Size-Exclusion Chromatography,* vol. 40, Elsevier, New York.
3. S. N. E. Omorodion, A. E. Hamielec, and J. L. Brash, in *Size Exclusion Chromatography,* T. Provder, Ed., ACS Symposium Series, 138, American Cehmical Society, Washington, DC.
4. K. J. Bombaugh, W. A. Dark, and J. N. Little, *Anal. Chem.,* **41,** 1337 (1969).
5. G. Glockner, *Polymer Characterisation by Liquid Chromatography*, vol. 34, Journal of Chromatography Library, Elsevier, Amsterdam, 1987, p. 170.
6. E. Meehan, in *Handbook of Size Exclusion Chromatography,* vol. 69, C. Wu, Eds., Marcel Dekker, New York, 1995, p. 25.
7. D. A. Brant and B. A. Burton, in *Solution Properties of Polysaccharides*, D. A. Brant, ACS Symposium Series, 150, American Chemical Society, Washington, DC, 1981.
8. P. S. Russo, M. Mustafa, T. Cao, and L. K. Stephens, *J. Colloid Interface Sci.,* **122,** 120 (1987).
9. A. C. M. Wu, A. Bough, E. C. Conrad, and K. E. Akien, *J. Chromatogr.,* **128,** 87 (1976).
10. S. Arcidiacono and D. L. Kaplan, *Biotechnol. Bioeng.,* **39,** 281 (1992).
11. R. G. Beri, J. Walker, E. T. Reese, and J. E. Rollings, *Carbohydr. Res., 238,* 11 (1993).
12. M. H. Ottoy, K. M. Varum, B. E. Christensen, M. W. Anthonsen, and O. Smidsred, *Carbohydr. Polym.,* **31,** 253 (1996).
13. M. Hasagawa, A. Isogai, and F. Ouabe, *Carbohydr. Res.,* **262,** 161 (1994).
14. A. M. Striegel and J. D. Tempa, *Int. J. Polym. Anal.Charact.,***2,** 213 (1996).
15. C. L. McCormick, P. A. Callais, and B. H. Hutchinson, *Macromolecules,* **18,** 2394 (1985).
16. A. Grubisic, P. Rempp, and H. A. Benoit, *Polym. Lett.,* **5,** 753 (1967).
17. M. L. Fishman, W. C. Damert, J. G. Phillips, and R. A. Barford, *Carbohydr. Res.,* **160,** 215 (1987).
18. T. Kato, T. Tokrya, and A. J. Takohoski, *J. Chromatogr.,* **256,** 61 (1983).
19. S. Nilsson, L. Sundelof, and B. Porsch, *Carbohydr. Polym.,* **28,** 265 (1995).
20. K. Jumal, S. E. Harding, J. R. Mitchell, K. M. To, I. Hayter, J. E. O'Mullane, S. Ward-Smith, *Carbohydr. Polym.,* **29,** 105 (1996).
21. A. Huber, *J. Appl. Polym. Sci., Appl. Polym. Symp.,* **48,** 95 (1991).
22. B. H. Zimm, J. Chem. Phys., **16,** 1099 (1948).
23. W. F. Reed, *Macromol. Chem. Phys.,* **196,** 1539 (1995).
24. C. Strazielle, in *Light Scattering from Polymer Solutions,* M. B. Huglin, Ed., Academic Press, New York, 1972.
25. W. W. Yau, J. J. Kirkland, and D. D. Bly, *Modern Size Exclusion Chromatography,* John Wiley & Sons, New York, 1979, p. 91.
26. W. B. Neely, *J. Polym. Sci., Part A,* **1,** 311 (1963).
27. W. B. Neely, *J. Am. Chem. Soc.,* **82,** 4354 (1960).
28. W. B. Neely, *J. Org. Chem.,* **26,** 3015 (1961).

29. W. Kuhn, P. Moser, and H. Majer, *Helv. Chim. Acta,* **44,** 770 (1961).
30. M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, *Anal. Chem.,* **28,** 350 (1956).
31. M. B. Mustafa, D. L. Tipton, M. D. Barkley, P. S. Russo, and F. D. Blum, *Macromolecules,* **26,** 370 (1993).
32. N. Robitaille, N. Turcotte, S. Fortin, and G. Charlet, *Macromolecules*, **24,** 2413 (1991).
33. Y. Ouano and W. Kaye, *J. Polym. Sci., Polym. Chem. Ed.,* **12,** 1151 (1974).
34. W. Radke and A. H. E. Muller, in *Strategies in Size Exclusion Chromatography*, M. Potschka and P. L. Dubin, Eds., ACS Symposium Series, 635, American Chemical Society, Washington, DC, 1992.
35. M. C. Wirick, *J. Polym. Sci., Part A-1,* **6,** 1705 (1968).
36. J. Reuben, Macromolecules, **17,** 156 (1984).
37. J. R. DeMember, *J. Appl. Polym. Sci.,* **21,** 621 (1977).
38. R. A. Gelman, *J. Appl. Polym. Sci.,* **27,** 2957 (1982).
39. E. K. Juast and T. G. Majewicz, in *Encyclopedia of Polymer Science and Technology,* 2nd ed., vol. 3, John Wiley & Sons, New York, p. 233.
40. J. Brandrup and E. H. Immergut, Eds., *Polymer Handbook,* 2nd ed., vol. 108, John Wiley & Sons, New York, 1975; M. B. Huglin, Ed., in *Light Scattering from Polymer Solutions,* Academic Press, New York, 1972.
41. H. G. Barth, E. I. du Pont de Nemours, personal communication.
42. R. S. Werbowyj and D. G. Gray, *Macromolecules,* **13,** 69 (1980).
43. Most of the older literature report *dn*/*dc* values at shorter λ. These data can be extrapolated to 632.8 nm using the Cauchy relationship to give values in good agreement with those reported here. See, for example, H. Vink and G. Dahlstrom, *Makromol. Chem.,* **109,** 249 (1967).
44. W. F. Reed, in *Strategies in Size Exclusion Chromatography*, M. Potschka and P. L. Dubin, ACS Symp. Series, 635, American Chemical Society, Washington, DC, 1996.
45. The validity of universal calibration using viscosity derived hydrodynamic volume as the definitive size parameter has been the subject of some dispute. See, for example, P. L. Dubin and J. M. Principi, *Macromolecules,* **22,** 1891 (1989).
46. Z. Bu, P. S. Russo, D. L. Tipton, and I. I. Negulescu, *Macromolecules,* **27,** 6871 (1994).
47. L. Mrkvicková, *Macromolecules,* **30,** 5175 (1997).
48. M. G. Wirick and M. H. Waldman, *J. Appl. Polym. Sci.,* **14,** 579 (1970).
49. R. D. Gilbert and P. A. Patton, *Prog. Polym. Sci.,* **9,** 115 (1983).