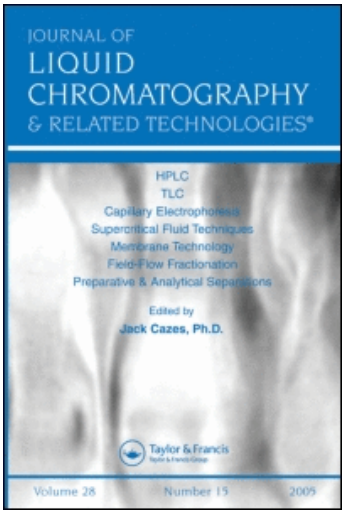

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SIZE-EXCLUSION CHROMATOGRAPHY USING MIXED-BED COLUMNS WITH DIMETHYLFORMAMIDE AT NEAR-AMBIENT CONDITIONS: COMPARISON OF μSTYRAGEL HT LINEAR AND PL GEL MIXED-BED COLUMNS

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ABSTRACT

Mixed-bed size-exclusion chromatography (SEC) columns packed with 10 μm particles are evaluated at near-ambient conditions using dimethylformamide (DMF) containing 0.01 M LiBr as the eluent. Two popular columns (HT Linear from Millipore Waters Chromatography Division and PL Gel from Polymer Laboratories) are evaluated in terms of operating pressure, resolution, fits to calibration data, and the accuracy of molecular weight distributions calculated for a broad, high-molecular-weight poly(methyl methacrylate) standard. Distinct differences in the operating characteristics between the columns are observed; however, it is shown that molecular-weight distributions comparable to those measured by SEC in a low-viscosity solvent (THF) can be obtained on both columns in DMF without the need to operate at elevated temperatures

INTRODUCTION

So-called "linear" or "mixed-bed" size-exclusion chromatography (SEC) columns contain mixtures of particles with different pore sizes. There are at least two advantages to these columns:

- They provide general-purpose column sets which cover a broad molecular-weight range. This avoids coupling columns of individual pore sizes, a practice that can result in unusual calibration curve shapes and discontinuities.

- Fewer columns are needed in inventory, since the columns in each set are supposedly identical and interchangeable.

These columns are frequently advertised to provide "linear" calibrations plots of logM versus retention volume, although this is often not the case; in some instances, the calibration curves are not well fitted even with third-order polynomials (1).

Many of the developments in new mixed-bed column technology have been directed toward smaller particle sizes (e.g., 5 μm). Many of these columns provide exceptional resolution in solvents such as tetrahydrofuran (THF), toluene, and dichloromethane at room temperature. However, we have found 5 μm particle-diameter mixed-bed columns unacceptable for work in viscous solvents at near-ambient conditions for at least four reasons:

- Operating pressures are high.
- The column exclusion limit is often below MW = 10^6, particularly for semirigid polymers in polar solvents.
- Shear degradation becomes a significant problem for high-molecular-weight polymers on 5 μm columns.
- Column lifetimes are unacceptably short.

For these reasons, larger particle diameters (e.g., 10 μm) retain a significant niche in SEC.

Some vendors recommend operating viscous solvents such as DMF at elevated temperatures. Various reasons have been given for high-temperature operation: reduced operating pressures, improved sample solubility, less sample adsorption, reduced shear degradation, and increased sample diffusion. There apparently is not universal agreement on these points when using DMF as an eluent. Conditions range between ambient and 135°C for polymers completely soluble in DMF at room temperature (2-5). In some of these cases, it is hard to rationalize the benefits of operating at elevated temperatures based on the above reasons. We suspect that most unreported work is at near-ambient conditions, since most workers prefer to avoid complicated and expensive high-temperature SEC equipment. This preference, in addition to the apparent confusion over the need to operate at elevated temperatures, makes clarification of near-ambient operation in DMF very appropriate.

In this paper, we examine the validity of near-ambient SEC using 10 μm mixed-bed columns in DMF/0.01 M LiBr. Lithium bromide is commonly added to DMF to suppress anomalous SEC effects observed for polar polymers (2, 6-10). Characteristics of two popular columns that are important to operation at these conditions are compared.

EXPERIMENTAL

Materials

Three μStyragel HT Linear columns, 7.8 mm i.d. x 300 mm, were supplied for evaluation by Millipore, Waters Chromatography Division, Milford, MA. Three 7.5 x 300 mm PL Gel 10 μm mixed-bed columns were purchased from Polymer Laboratories, Amherst, MA. Nominal particle diameters of both materials are 10 μm; exact particle diameters and particle size distributions are not supplied by the vendors. HPLC-grade DMF (OmniSolve, EM Laboratories) was used without further purification. Lithium bromide was obtained from Aldrich Chemical Company, Inc. (Milwaukee, WI).

Column and Eluent Preparation

Three PL Gel columns were converted from THF at room temperature by first passing at 0.1 mL/min three column volumes of acetone, followed by three column volumes of DMF. The μStyragel HT Linear columns were supplied in methyl ethyl ketone. They were converted directly from this solvent at room temperature by passing at 0.1 mL/min three column volumes of DMF (6). DMF/0.01 M LiBr was passed through a 0.5 μm Teflon® filter, degassed under vacuum, and sparged continuously during use. Both column sets were converted from pure DMF to DMF/ 0.01 M LiBr at 1.0 mL/min. A minimal flow (e.g., 0.05 mL/min) was maintained when the columns were not in use for periods up to one week. When columns were not used for periods longer than one week, they were flushed with DMF without LiBr and plugged.

Column Evaluation

Narrow-molecular-weight-distribution poly(methyl methacrylate)s (PMMA) were purchased from Polymer Laboratories (Amherst, MA). Standards were injected individually in a volume of 100 μL, containing 0.1% (v/v) acetone as a pulse marker. Concentrations of standards were 0.1 mg/mL for PMMA's greater than 800K, 0.4 mg/mL for PMMA 200K-800K, 1.0 mg/mL for PMMA 49K-100K, 1.5 mg/mL for PMMA 15K-25K, and 2.0 mg/mL for PMMA less than 10K. Nominal flowrates were 1.0 mL/min throughout. The actual flow rate during the elution of the first standard was calculated from the time needed to collect 10 mL of eluent in a calibrated 10 mL volumetric flask, and the flow rates of subsequent injections were corrected by using the retention time of the acetone pulse marker. Broad-molecular-weight-distribution PMMA, synthesized by free-radical polymerization by Kodak Laboratory and Research Products, was injected at a concentration of 2.0 mg/mL in a volume of 100 μL of eluent to which was added 0.1% acetone by volume. The broad PMMA standard was characterized by narrow-standard SEC, universal calibration, and low-angle laser light scattering (LALLS) in THF by a multidetector SEC

arrangement described elsewhere (1,12). Intrinsic viscosities in DMF were obtained by conventional capillary tube viscometry and with a Model 110 differential viscometry detector (Viscotek Corp., Porter, TX) connected in parallel with a differential refractive index (DRI) detector. The columns and all detectors were thermostated to 35°C.

RESULTS AND DISCUSSION

Approximately 30,000 theoretical plates were measured on both column sets from acetone peaks obtained from 100 µL injections of 0.1% acetone in DMF/0.01 M LiBr. Although this value is impressive for 10 µm particle-diameter columns, the efficiency of small-molecule elution is not necessarily a good indicator of SEC column performance. We are particularly interested in operating characteristics and the quality of calibration for polymers in viscous solvents, not small molecules such as acetone. These characteristics include

Operating Pressure

Three µStyragel HT Linear columns generate 300 psi of pressure in our system, including detectors. This is actually lower than pressures obtained with low-viscosity solvents on many 10 µm particle-diameter SEC columns. In comparison, three PL Gel mixed-bed columns operate at approximately 1500 psi in the same configuration. Part of this difference may be attributed to the smaller diameter of PL Gel mixed-bed columns (7.5 versus 7.8 mm); smaller column diameters result in higher eluent linear velocities at equivalent volumetric flow rates, thus producing higher pressures; however, differences in column diameters cannot account completely for the large difference in operating pressures between column sets. HT Linear columns are apparently more permeable to DMF/0.01 M LiBr than are PL Gel columns. Generally, lower pressures are preferred in SEC.

Fits to Calibration Data

Linear fits of narrow, PMMA standard calibration curves are shown in Figure 1. Retention volumes are larger on the wider µStyragel columns. If linearity is of importance, it is evident that this criterion is better fulfilled by the µStyragel HT Linear columns. We do not view this as a significant advantage, however, because SEC calibration data are commonly fitted with a third-order polynomial (Figure 2). We have shown previously that the quality of fits to calibration data can be assessed in terms of percent error in molecular weight, M, by analysis of residuals (1), where

$$\text{Residual (\% Error in M)} = \frac{100(M_{fit} - M_{std})}{M_{std}} \qquad (1)$$

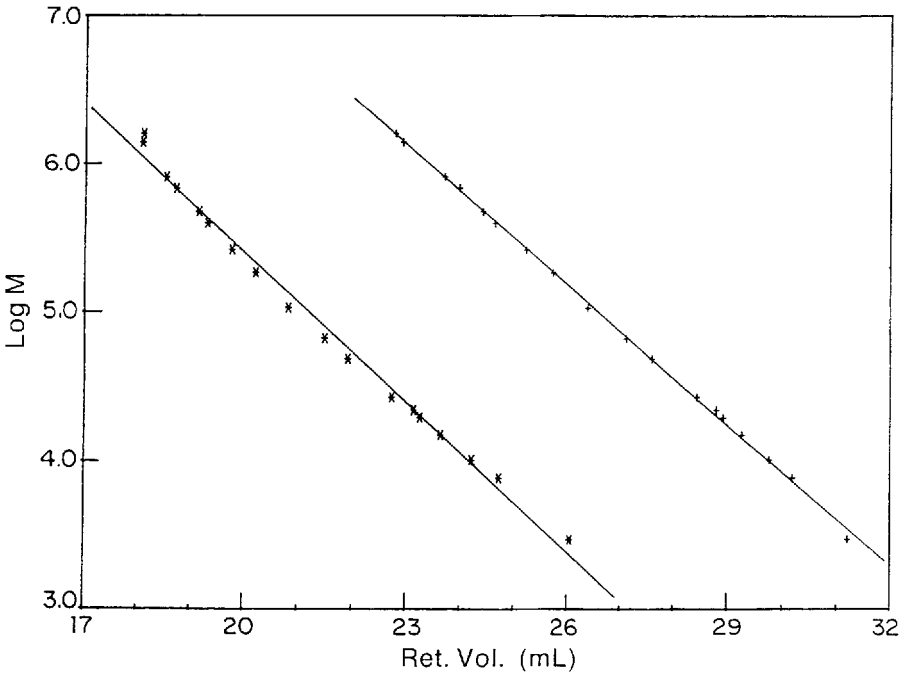

FIGURE 1. Linear fits to PMMA narrow standard data in DMF/0.01 M LiBr for (a) three PL Gel 10 μm mixed-bed columns and (b) three μStyragel HT Linear columns.

is a measure of the goodness of fit. A random scatter of residuals close to and about zero is optimum. Analyzed in this fashion (Figures 3 and 4), we find that molecular-weight calibration data from both columns are fitted equally well with third-order polynomials. A third-order polynomial clearly provides a better fit to PL gel column data than a simple linear fit (Figure 4), while a smaller but significant difference is seen in HT Linear columns (Figure 3). Of significance is the nearly random distribution of residuals for third-order fits in both column sets.

We cannot overlook the possibility that plots of residuals may be affected by incorrect molecular-weight values quoted by the vendor. We have shown that molecular-weight-sensitive detection can be used effectively to assess SEC calibration curves, independent of data on standards supplied by vendors (1). Either weight-average molecular weights measured by LALLS or

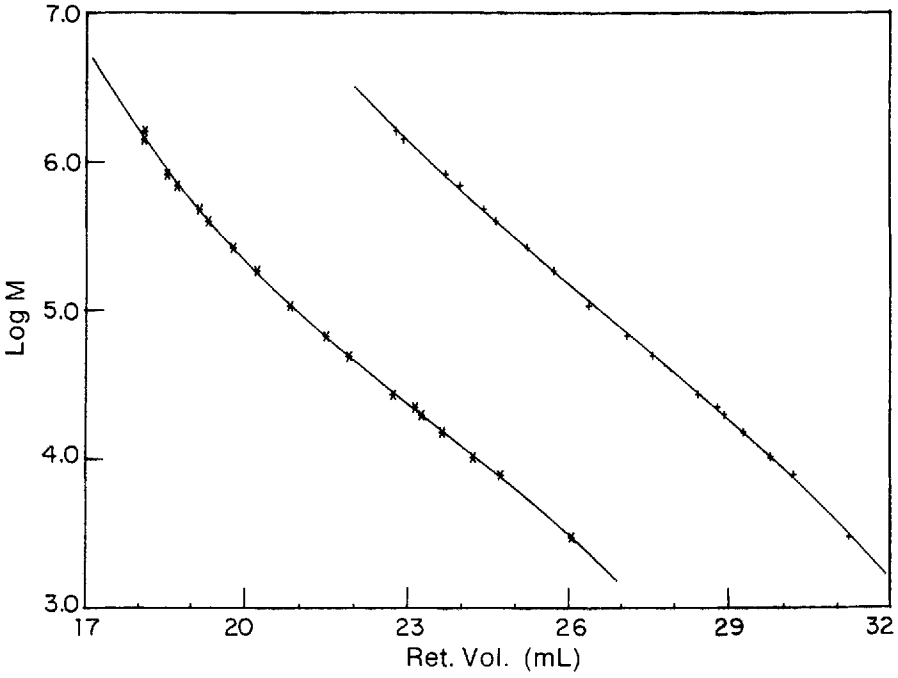

FIGURE 2 Third-order polynomial fits to PMMA narrow standard data in DMF/0.01 M LiBr for (a) three PL Gel 10 μm mixed-bed columns and (b) three μStyragel HT Linear columns.

intrinsic viscosities measured by on-line viscometric detection may be used. Residuals of third-order fits to intrinsic viscosity data, [η],

$$\text{Residual (\% Error in } [\eta]) = \frac{100([\eta]_{fit} - [\eta]_{std})}{[\eta]_{std}} \qquad (2)$$

obtained by viscometry detection (Figure 5) are convincingly random about zero, again confirming that reasonable fits of calibration data can be obtained. In contrast, pronounced winding has been reported previously on 5 μm columns, which was shown to adversely affect the calculation of molecular-weight averages (1). We note that the quality of third-order fits to calibration data on both 10 μm mixed-bed columns in this study is better than data obtained previously on 5 μm columns. This is a desirable feature over so-called "high efficiency" 5 μm mixed-beds.

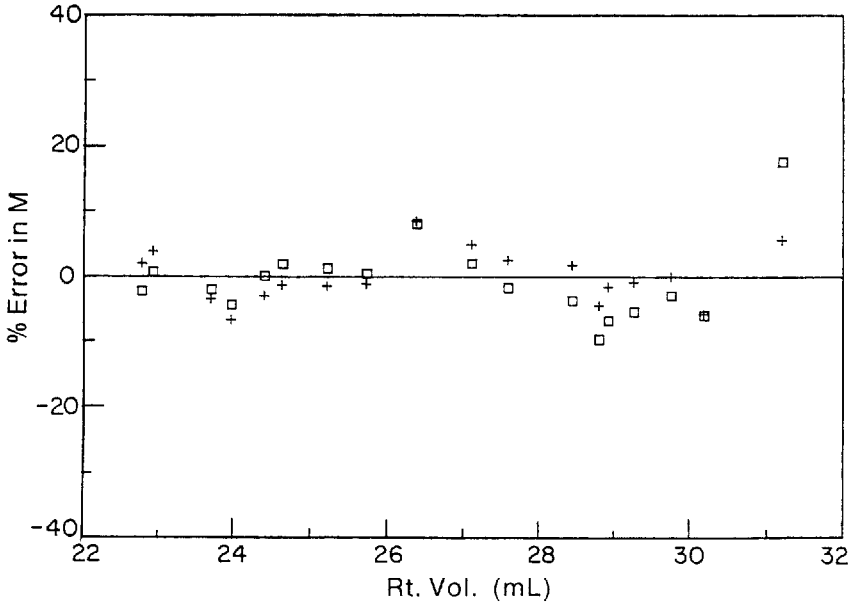

FIGURE 3. Percent error in M for PMMA narrow standard data obtained on three μStyragel HT Linear columns in DMF/0.01 M LiBr for □ linear fit and + third order polynomial fit.

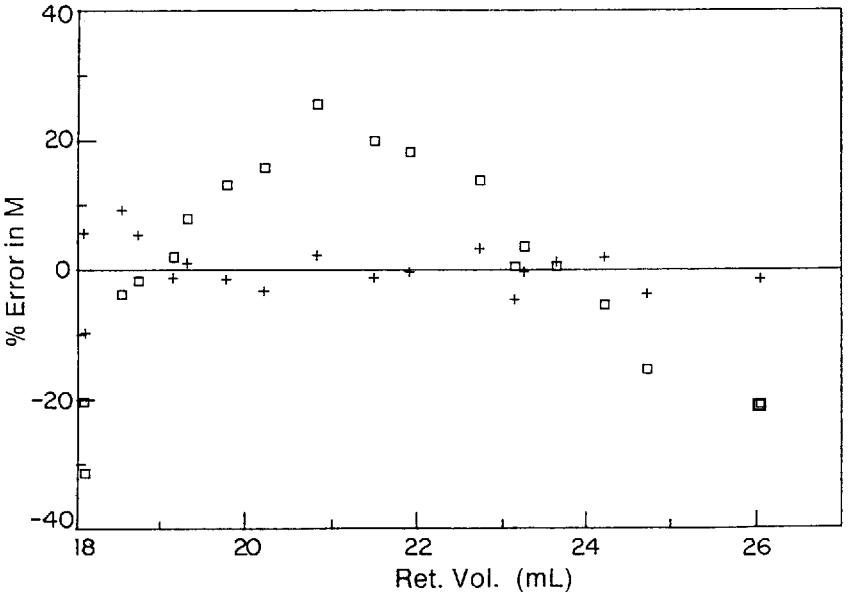

FIGURE 4. Percent error in M for PMMA narrow standard data obtained on three PL Gel mixed-bed columns in DMF/0.01 M LiBr for □ linear fit and + third order polynomial fit.

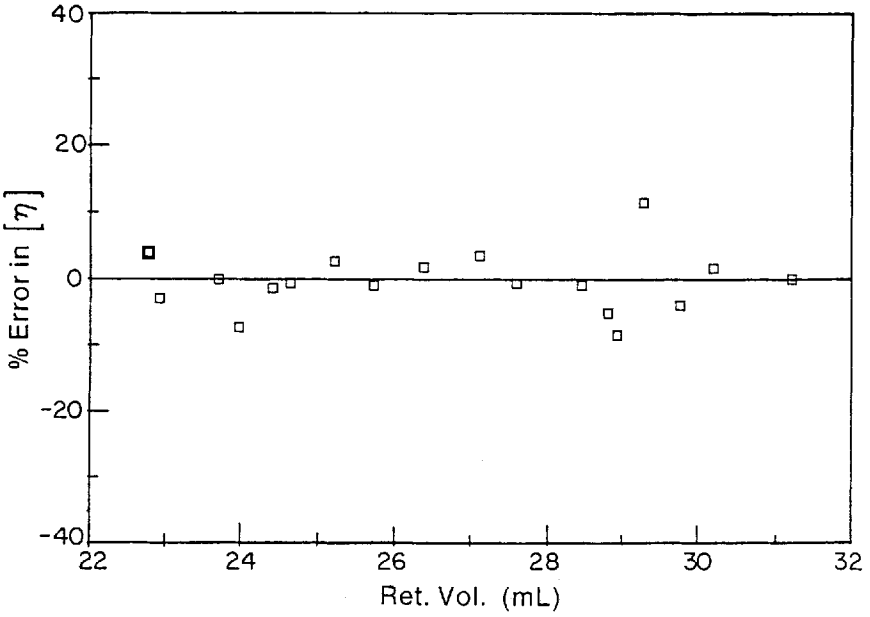

FIGURE 5. Percent error in [η] for third-order polynomial fit to PMMA narrow standard data using viscometry detection and three μStyragel HT Linear columns. Conditions are the same as for Figures 1-4.

Resolution

Several methods have been proposed for the assessment of resolution in SEC (e.g., see discussion in Chapter 3 of 14). We have chosen the resolution index, T, proposed by Glockner (15) because it has an easily understood physical meaning. It is the ratio of molecular weights which can be completely separated, assuming Gaussian curves.

$$T = \left[\frac{M_1}{M_2}\right]^{1/R_{S,CORR}} \tag{3}$$

The corrected resolution, $R_{S,CORR}$,

$$R_{S,CORR} = \frac{t_{R2} - t_{R1}}{0.5\left[\frac{w_{S1}}{d_1} + \frac{w_{S2}}{d_2}\right]} \tag{4}$$

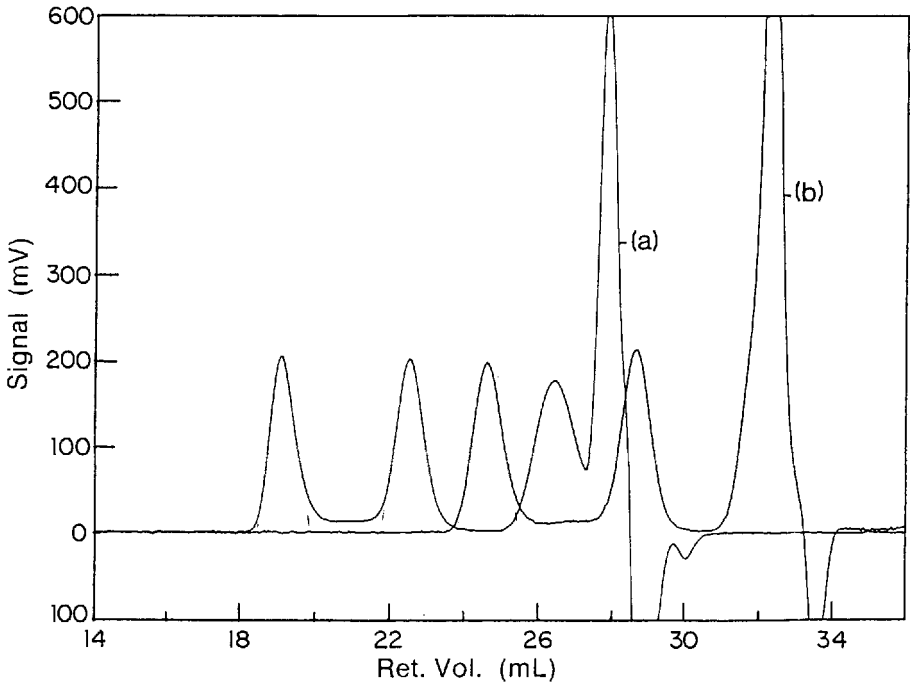

FIGURE 6. Chromatograms of PMMA 600,000, 35,000 and 2,000 in DMF/0.01 M LiBr on (a) three PL Gel mixed-bed columns and (b) three μStyragel HT Linear columns.

is calculated from retention times, t_{R1} and t_{R2}, and peak widths, w_{S1} and w_{S2}, of narrow-molecular-weight-distribution standards with molecular weights M_1 and M_2. The resolution is "corrected" for the known polydispersities (M_w/M_n) of each standard, d_1 and d_2. Calculated this way, resolution is comparatively insensitive to the sample selected. From a number of narrow standards between MW 10^4-10^6, we calculate $T = 3.15 \pm 0.05$ for PL gel mixed-bed columns, and $T = 2.93 \pm 0.14$ for HT Linear columns. The difference is not significant over this molecular-weight range. Of note, however, is the difference in resolution at molecular weights less than 10^4. HT Linear columns do not resolve PMMA 2K from the first system peak (Figure 6), indicating that the HT Linear calibration curve is turning downward more steeply in the low-molecular-weight region than that for PL Gel mixed-bed columns.

Molecular Weight Distributions of a Broad Standard

The most relevant measure of column performance is the ability to calculate accurate molecular-weight distributions. Because of our concerns for nonideal SEC behavior caused by

TABLE 1

PMMA Broad Standard Molecular Weight Averages[a]

	M_n	M_w	M_z
THF			
Narrow Std.			
5 μm	169,000 ± 5,000	437,000 ± 7,000	806,000 ± 29,000
10 μm	141,000 ± 8,000	426,000 ± 8,000	830,000 ± 23,000
Univ. Cal.	147,000 ± 10,000	434,000 ± 13,000	858,000 ± 17,000
LALLS	172,000 ± 20,000	443,000 ± 19,000	828,000 ± 35,000
DMF/0.01 M LiBr			
Narrow Std. Cal.			
HT Linear	162,000 ± 7,000	430,000 ± 8,000	845,000 ± 29,000
PL Gel	166,000 ± 6,000	428,000 ± 7,000	855,000 ± 28,000
		[η] (dL/g)	
DMF/0.01 M LiBr Viscosity Detection		0.818	
DMF/0.01 M LiBr Capillary		0.819	

[a]Error reported as 1 sigma std. dev.

high eluent viscosity, we purposely chose a broad PMMA standard which contains a high-molecular-weight tail. It is assumed that anomalous behavior will most likely be observed in a high-molecular-weight polymer, where shear degradation and small polymer-diffusion coefficients may be of concern. SEC of PMMA in low-viscosity solvents, particularly THF, has been documented extensively and is presumably well behaved. Molecular-weight averages obtained on high-resolution columns in THF using molecular-weight-sensitive detectors (1), corrected for axial dispersion (15) are given in Table 1. We accept these values as our best estimate of the "true" molecular-weight averages. Also included are molecular-weight averages measured in THF on 10 μm PL Gel mixed-bed columns, and by universal calibration and LALLS in THF on 5 μm columns. We see that, even in this "well-behaved" SEC, differences in molecular-weight averages will be observed, although all values are statistically equivalent, based on experimental error from multiple analyses.

Molecular-weight averages obtained by narrow-standard calibration in DMF/0.01 M LiBr are essentially identical for both HT Linear and PL Gel columns. We also note that they are in

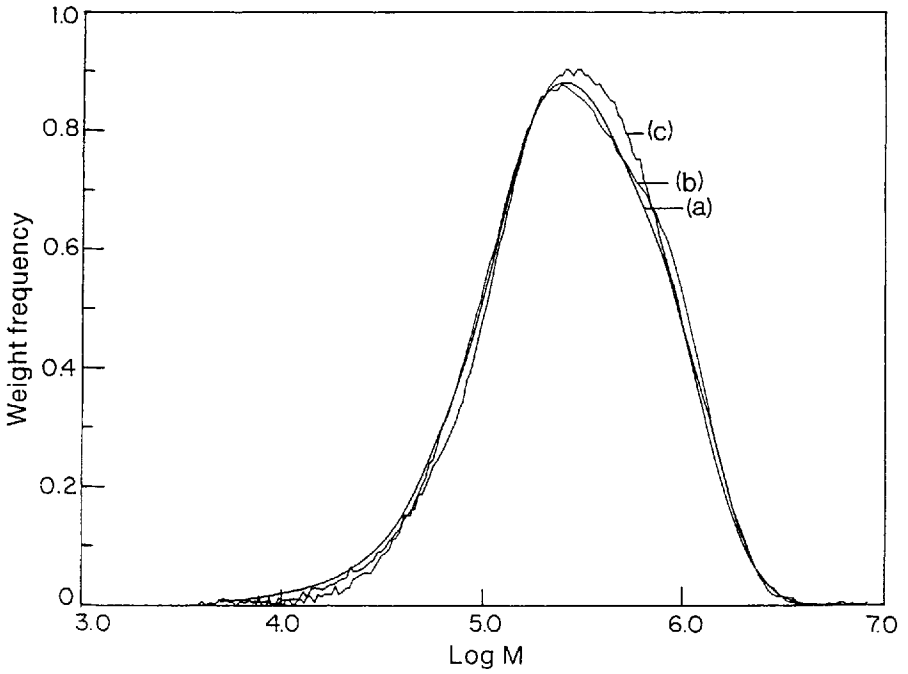

FIGURE 7. Molecular-weight distributions of broad PMMA obtained by narrow-standard calibration (a) in THF on three PL Gel 10 μm mixed bed columns, (b) in DMF/0.01 M LiBr on three PL Gel 10 μm mixed bed columns, and (c) in DMF/0.01 M LiBr on three μStyragel HT Linear columns.

statistical agreement with averages measured in THF. However, the molecular-weight averages in Table 1 provide comparison of only three points along the molecular-weight distribution. Although these commonly reported values do not show significant differences between the columns and different solvents, this does not exclude the presence of differences in the entire distribution. Entire molecular-weight distributions obtained on 10 μm mixed-bed columns in THF and DMF in Figure 7 indeed are not superimposable. Distributions obtained on PL Gel columns in THF and DMF are quite similar, as might be expected for distributions obtained on identical columns in different solvents. The distribution obtained on HT Linear columns differ slightly in shape from the distribution obtained in THF, particularly at high molecular weights. In all cases the differences are small and could well be acceptable for most purposes.

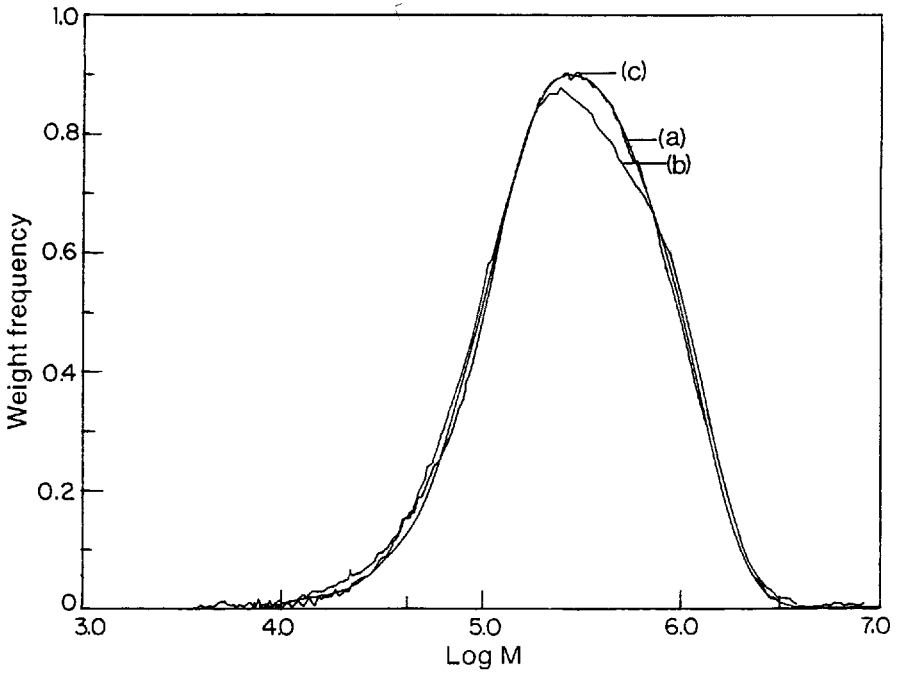

FIGURE 8. Molecular-weight distributions of broad PMMA obtained (a) by LALLS in THF on three PL Gel 10 μm mixed bed columns, (b) by narrow-standard calibration in DMF/0.01 M LiBr on three PL Gel 10 μm mixed bed columns, and (c) by narrow-standard calibration in DMF/0.01 M LiBr on three μStyragel HT Linear columns.

We have no immediate method of determining which molecular-weight distribution is "correct" from molecular-weight distributions calculated by narrow-standard calibration (Figure 7). The shapes of such plots depend on subtle differences in third-order polynomial fits to the calibration data. In addition, the calibration curve must accurately approximate the true exclusion profile of a mixed-bed column set, including small discontinuities unique to each vendor's manufacturing and packing processes. These discontinuites may not be apparent unless the calibration curve is defined by a large number of narrow standards. Alternatively, we can compare distributions determined by narrow-standard calibration with the absolute molecular-weight distribution measured by LALLS in THF. Using LALLS, the molecular-weight distribution is independent of the number and quality of quoted values of narrow standards, as well as the quality of fits to calibration data. In Figure 8, we observe that HT Linear columns provide the best approximation of the LALLS molecular-weight distribution at high molecular weights. Neither

column approximates the distribution obtained by LALLS at low molecular weights. This is a result of inaccuracies in LALLS analyses; light-scattering signals are weak at low molecular weights and this region of the molecular-weight distribution must be obtained by extrapolation.

Using viscometry detection, we measured a Mark-Houwink exponent a = 0.674, and coefficient K = 0.000132 dL/g for PMMA standards between 10,000 and 1,400,000 in DMF/0.01 M LiBr at 35°C. Molecular weight averages calculated by viscometry detection and universal calibration are in agreement with those obtained by other means in THF and DMF. In addition, bulk intrinsic viscosities measured by SEC/viscometry detection and capillary viscometry are equivalent (Table 1). The agreement of results in THF and DMF in all cases indicate that the comparatively high viscosity of DMF at near-ambient temperature does not adversely affect SEC results for this PMMA sample. We would anticipate greater discrepancies in molecular-weight distributions at high molecular weights if factors such as shear degradation or restricted diffusion were important. This finding is not completely unexpected; Mori (17) saw no change in SEC retention volumes in THF upon the addition of up to 5.8% of low-molecular-weight PEG to the mobile phase and concluded that eluent viscosity had no significant effect on SEC behavior.

CONCLUSIONS

This study demonstrates that SEC results similar to those obtained in a low-viscosity solvent such as THF can be obtained in DMF/0.01 M LiBr at near-ambient conditions for relatively high-molecular-weight PMMA. Differences observed in molecular-weight distributions obtained in THF and DMF/0.01 M LiBr are attributed to inadequacies inherent in narrow-standard calibration rather than anomalous SEC behavior caused by the high viscosity of DMF. Although we have not exhaustively evaluated SEC behavior of other polymers, we have not seen any advantage to high-temperature operation in DMF/0.01 M LiBr unless necessitated by sample solubility or evidence for sample adsorption.

We find that 10 μm mixed-bed columns can provide acceptable results, and that some columns (μStyragel HT Linear) operate at low pressures, even in viscous solvents such as DMF/0.01 M LiBr. These columns show no obvious shear degradation of our PMMA sample in the 10^3-10^6 molecular-weight range and provide accurate measures on M_n, M_w, and M_z by narrow-standard calibration. We feel that further improvements in the accuracy of molecular-weight distributions will be obtained by refinements in calibration rather than by changes in operating conditions, particularly operating temperature.

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