Molar Mass Distributions of Polymers from Size Exclusion Chromatography and Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry: Methods for Comparison

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ABSTRACT: Equations are presented for calculating molar mass averages and molar mass distributions from matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) data and from size exclusion chromatography (SEC) data. The utility of polydispersity is examined as an indicator of the expectation of MALDI-TOF MS mass discrimination effects. Cumulative distributions are found to be rich in information for comparing the two techniques and are easily obtained from both SEC and MALDI-TOF MS data. Analyses of a series of narrow molar mass distribution poly(methyl methacrylate) (PMMA) standards and one polydisperse sample have been performed with both methods. MALDI-TOF MS did not detect dimer and trimer in the PMMA samples, and it often indicated lower amounts of high-molar-mass polymers than did SEC. The results showed that the distribution breadth, as evidenced by the standard deviation of the distribution (calculated from the polydispersity and number-average molar mass), correlated well with the molar mass range observed in the MALDI-TOF MS spectra, whereas the polydispersity alone did not. Ratioing the extremes in the molar mass concentrations measured with the SEC differential refractometer, which were necessary to adequately define molar mass distributions, showed that detector dynamic range values as high as approximately 370,000 were required for the polydisperse samples. © 2005 Wiley Periodicals, Inc. J Appl Polym Sci 97: 627–639, 2005

Key words: MALDI; mass spectrometry; molar mass distribution

INTRODUCTION

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is being increasingly used for the determination of synthetic polymer molar mass distributions.[1–3] The information obtained from MALDI-TOF MS is, in many ways, complementary to information from size exclusion chromatography (SEC), which has been the primary method for measuring molar mass distributions for several years. For example, MALDI-TOF MS spectral intensities are proportional to the number of molecules of mass M, whereas SEC uses concentration detectors that are sensitive to the weight of polymer of mass M. MALDI-TOF MS can provide detailed structural information, such as end groups, whereas SEC can distinguish and quantify polymer chain conformations and dilute solution properties. Both methods have mass ranges that can extend from a few hundred daltons to greater than 10^6 Da, although SEC viscometry and light scattering detectors have increasing sensitivity with increasing molar mass, whereas the MALDI-TOF MS signal-to-noise (S/N) ratio usually worsens with increasing molar mass. MALDI-TOF MS can measure the mass of individual oligomers with an accuracy of <1 Da; the mass accuracy of SEC is generally limited to 1–2%, depending on the peak molar mass, M_p values of narrow standards or on the calibration of molar mass-sensitive detectors. Conversely, SEC detectors are advantageous for determining the concentrations and masses of molecules over a large mass range; MALDI-TOF MS intensities may be affected by mass discrimination, and baselines are more difficult to select than those for SEC chromatograms. There is no simple answer to the question of which method is correct. Because of the complementary nature of the techniques and their relative strengths and weaknesses, the fair answer is probably neither, largely because it depends on the nature of the test samples and the methods of comparison.

Most comparisons of MALDI-TOF MS and SEC have focused on the number-average molar mass

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(M_n), the weight-average (M_w), and the M_w/M_n ratio, which is commonly called polydispersity.[4–11] Procedures have been proposed that manipulate MALDI-TOF MS spectra to give the correct values of M_n and M_w calculated by SEC.[6,12–14] The possible inadequacy of comparing M_n and M_w was recognized in an interlaboratory comparison of MALDI-TOF MS polystyrene molar mass distributions,[15] which also considered the *z*-average molar mass (M_z) and the reciprocal moments $M_{1/n}$, $M_{1/w}$, and $M_{1/z}$. It was suggested that even these additional averages may not adequately represent the low-molar-mass and high-molar-mass tails of distributions, and dividing the MALDI-TOF MS molar mass distributions into 11 bins was better for quantitative comparisons.

Similar concerns may have prompted other workers to compare SEC and MALDI-TOF MS molar mass distributions graphically by transforming the data sets to common linear mass or logarithmic mass scales.[14,16] Comparisons of the entire molar mass distributions provide considerably more information than just molar mass averages. Relationships between MALDI-TOF MS spectra and SEC chromatograms,[17] as well as interconversions of the MALDI-TOF MS number and SEC weight molar mass distributions on linear and logarithmic scales,[16] have been described. These transformations are essential because a simple visual comparison of matrix-assisted laser desorption/ionization (MALDI) spectra and SEC chromatograms can be quite deceiving. For example, Jackson et al.[18] pointed out that the most probable peak values for MALDI will always be lower than those for SEC and are a function of how the data are displayed. Also, normalized SEC and MALDI-TOF MS molar mass distributions can be difficult to superimpose and compare quantitatively because of the discrete nature of MALDI-TOF MS distributions and the continuous nature of SEC distributions.

Understandably, comparisons between the techniques can be confusing and subject to interpretation. For this reason, this work provides a summary of the relationships between molar mass distributions obtained by SEC and MALDI-TOF MS and presents examples of how the two results can be used in a complementary way to improve results from both methods.

THEORY

Differential molar mass distributions

Molar mass distributions from SEC are usually plotted as the normalized weight fraction of material, $W_N(\log M)$, versus $\log M$, where $W_N(\log M)d \log M$ is the weight fraction of polymer from $\log M$ to $\log M + d \log M$. This is termed a differential molar mass distribution on a mass basis. However, molar mass distributions from MALDI-TOF MS are more often computed as $n_N(M)$ versus M, where $n_N(M)dM$ is the molar fraction of polymer from M to $M + dM$. Here, we term this a differential molar mass distribution on a number basis. The interrelationship between these two types of distributions is demonstrated in this section.

The weight fraction of polymer in a sample between molar masses M and $M + dM$ should be the same as the weight fraction between $\log M$ and $\log M + d \log M$:

$$W_N(M)dM = W_N(\log M)d \log M \tag{1}$$

Equations (2) and (3) can be used to convert the normalized ordinate values, $W_N(\log M)$, of SEC distribution (i.e., the weight fraction of the polymer from $\log M$ to $\log M + d \log M$ per $\log M$ increment, $d \log M$) to $W_N(M)$ (the weight fraction of polymer from M to $M + dM$ per M increment, dM). Therefore,

$$W_N(M) = W_N(\log M) \frac{d \log M}{dM} \tag{2}$$

$$W_N(M) = W_N(\log M) \frac{1}{\ln(10)\, M} \tag{3}$$

The number of moles of polymer between M and $M + dM$ per unit of mass injected is

$$n(M)dM = \frac{W_N(M)dM}{M} = \frac{W_N(\log M)d \log M}{M} \tag{4}$$

The molar fraction per dM increment, $n_N(M)$, is obtained from

$$n_N(M) = \frac{\dfrac{W_N(M)}{M}}{\displaystyle\int_0^\infty \frac{W_N(M)}{M}\, dM} = \frac{\dfrac{W_N(\log M)}{M^2 \ln(10)}}{\displaystyle\int_0^\infty \frac{W_N(\log M)d \log M}{M}} \tag{5}$$

Equation (5) can be used to convert differential molar mass distributions on a mass basis [$W_N(M)$ vs M or $W_N(\log M)$ vs $\log M$] to differential molar mass distributions on a molar fraction basis.

Differential molar mass distributions on a molar fraction basis from SEC chromatograms

For an SEC differential refractive index (DRI) detector,

$$W_N(M)dM = -W_N(v)dv \tag{6}$$

where $W_N(v)$ is the normalized height of the DRI chromatogram at retention volume v and the proportionality constant relating detector response and mass is assumed to not be a function of molar mass. So, eq. (5) becomes

$$n_N(M) = \frac{\frac{W_N(\nu)}{M}\frac{d\nu}{dM}}{\int_0^\infty \frac{W_N(\nu)}{M}\,d\nu} = \frac{\frac{W_N(\nu)}{M^2\ln(10)}\frac{d\nu}{d\log M}}{\int_0^\infty \frac{W_N(\nu)d\nu}{M}} \quad (7)$$

Differential molar mass distributions on a molar fraction basis from MALDI-TOF MS spectra

MALDI-TOF MS spectra are commonly represented on a linear mass scale; the area of peaks is proportional to the number of molecules at a given mass-to-charge ratio (m/z). We assume that ions are singly charged so that MALDI-TOF MS m/z is equivalent to molar mass M. We also assume that both MALDI-TOF MS and SEC provide detector signals that vary continuously with the number and mass of molecules, respectively.

For MALDI-TOF MS intensities $h(M)$, where $h(M)dM$ is proportional to the number of molecules from M to $M + dM$, $n(M)dM$ can be converted to the heights of the differential molar mass distribution on a molar fraction basis:

$$n_N(M) = \frac{k_n(M)h(M)}{\int_0^\infty k_n(M)h(M)\,dM} \quad (8)$$

where $k_n(M)$ is the proportionality constant and is, in general, a function of molar mass. If the variation of $k_n(M)$ is assumed to be insignificant over the breadth of the molar mass distribution of the particular sample, the proportionality constants cancel, and eq. (8) can be written as follows:

$$n_N(M) = \frac{h(M)}{\int_0^\infty h(M)\,dM} \quad (9)$$

This assumption will be implicit in the remainder of the equations presented.

Differential molar mass distributions on a mass basis from MALDI-TOF MS spectra

The weight of molecules from M to $M + dM$, $W(M)dM$, is given by

$$W(M)\,dM = k_n h(M)M\,dM \quad (10)$$

The weight fraction per dM increment, $W_n(M)$, is obtained from

$$W_N(M) = \frac{h(M)M}{\int_0^\infty h(M)M\,dM} \quad (11)$$

The weight fraction per $d\log M$ increment on a log M scale is, therefore,

$$W_N(\log M) = \ln(10)W_N(M)M \quad (12)$$

Cumulative distributions

It is relatively easy to obtain a differential distribution from an SEC chromatogram. However, accomplishing this task with a MALDI-TOF MS is complicated by the fact that the spectra have discrete character: there are significant portions of the spectra between peaks that contain only noise. Ordinate values of a MALDI-TOF MS differential distribution are often much larger than those of the differential SEC distribution because the heights of those portions of the spectrum between discrete molar masses are negligibly small. Although it is likely that the MALDI-TOF MS detector is a continuous detector, in that eq. (8) is valid, unlike in SEC, the signals only appear across sequential, narrow ranges of molar mass. This is evident in the molar mass distribution of narrow-distribution poly(methyl methacrylate) (PMMA; PMMA 2990; see Fig. 1), for which both curves have unit area. Some workers have plotted these distributions by arbitrarily scaling the ordinate of one data set; this allows a visual comparison of the distributions. This has limited utility for quantitative comparisons. In such instances, cumulative distributions are useful.

The cumulative distributions from SEC are obtained as follows:

$$W_{N,\mathrm{cum}}(\log M) = \int_0^{\log M} W_N(\log M)d\log M \quad (13)$$

$$W_{N,\mathrm{cum}}(M) = \int_0^M W_N(M)dM \quad (14)$$

$$n_{N,\mathrm{cum}}(M) = \int_0^M n_N(M)dM \quad (15)$$

where $W_{N,\mathrm{cum}}(\log M)$ is the weight fraction of polymer of log molar mass less than (or equal to) log M, $W_{N,\mathrm{cum}}(M)$ is

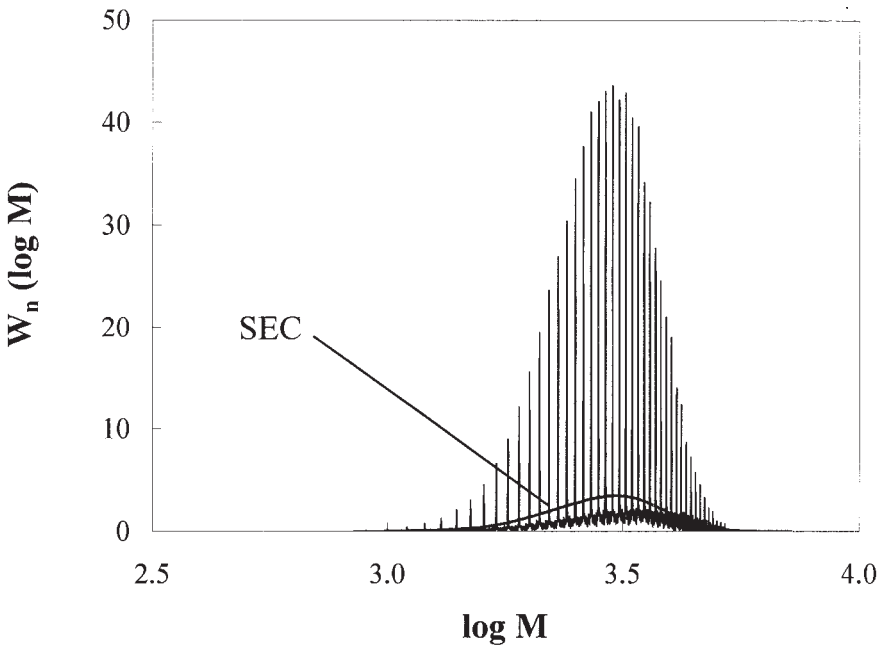

Figure 1 Differential molar mass distributions of PMMA 2990 obtained from MALDI and SEC.

the mass fraction of polymer of molar mass less than (or equal to) M, and $n_{N,\mathrm{cum}}(M)$ is the molar fraction of polymer of molar mass less than (or equal to) M.

Cumulative molar mass distributions on a number basis can be obtained from a MALDI-TOF MS spectra as follows:

$$n_{N,\mathrm{cum}}(M) = \int_0^M n_N(M)\, dM = \frac{\int_0^M h(M)\, dM}{\int_0^\infty h(M)\, dM} \qquad (16)$$

where $n_N(M)dM$ is the molar fraction of polymer from M to $M + dM$, $n_{N,\mathrm{cum}}(M)$ is the molar fraction of polymer of molar mass less than (or equal to) M, and, as before, $h(M)$ is the height of the MALDI-TOF MS signal at M.

The cumulative molar mass distribution, $W_{N,\mathrm{cum}}(M)$, versus M is obtained as follows:

$$W_{N,\mathrm{cum}}(M) = \frac{\int_0^M Mh(M)\, dM}{\int_0^\infty Mh(M)\, dM} \qquad (17)$$

where $W_{N,\mathrm{cum}}$ is the weight fraction of polymer of molar mass less than M. This can also be expressed in terms of log M:

$$W_{N,\mathrm{cum}}(\log M) = \frac{\int_0^M Mh(\log M) d \log M}{\int_0^\infty Mh(\log M) d \log M} \qquad (18)$$

where $W_{N,\mathrm{cum}}(\log M)$ is the weight fraction of polymer of log molar mass less than log M.

Molar mass averages

By definition, molar mass averages can be defined in terms of either the weight or number of moles of polymer:

$$M_k = \frac{\int_0^\infty M^k W_N(M)\, dM}{\int_0^\infty M^{k-1} W_N(M)\, dM} = \frac{\int_0^\infty M^{k+1} n_N(M)\, dM}{\int_0^\infty M^k n_N(M)\, dM} \qquad (19)$$

where k is 0, 1, and 2 for M_n, M_w, and M_z, respectively. A potentially useful relationship between $n_N(M)$, $n(M)$, and M_n for the whole polymer can be obtained as follows:

$$n_N(M) = \frac{\frac{W_N(M)}{M}}{\int_0^\infty \frac{W_N(M)}{M} dM} \frac{\int_0^\infty W_N(M)\, dM}{\int_0^\infty W_N(M)\, dM} \qquad (20)$$

because

$$\int_0^\infty W_N(M)\, dM = 1 \tag{21}$$

and because

$$M_n = \frac{\int_0^\infty W_N(M)\, dM}{\int_0^\infty \frac{W_N(M)}{M}\, dM} \tag{22}$$

Therefore,

$$n_N(M) = \frac{W_N(M)}{M} M_n = n(M) M_n \tag{23}$$

Polydispersity as a measure of the distribution breadth

Recent MALDI literature has recognized that molar mass diversity in a sample analyzed by MALDI is undesirable because the detector response constant, k_n, as well as other effects, can vary with the molar mass, rather than being constant, as quantitative interpretation normally assumes. Polydispersity, M_w/M_n, has been used as a measure of this diversity, with a low value of polydispersity recommended for avoiding such mass discrimination effects. However, a more widely recognized measure of the width of a distribution is its standard deviation. Following a derivation by Rudin[19] and considering continuous distributions instead of discrete distributions, we can define the square of the standard deviation (i.e., the variance), σ_n^2, of a number distribution, $n_N(M)$, versus M as follows:

$$\sigma_n^2 = \int_0^\infty n_N(M)\, (M - M_n)^2 dM \tag{24}$$

That is,

$$\sigma_n^2 = \int_0^\infty n_N(M) M^2\, dM - 2M_n \int_0^\infty n_N(M) M\, dM + M_n^2 \int_0^\infty n_N(M)\, dM \tag{25}$$

Because the area under $n_N(M)$ versus M is unity and by the definition of M_n and M_w [eq. (19)],

$$\sigma_n^2 = M_w M_n - {M_n}^2 \tag{26}$$

or

$$\sigma_n = \left[\left(\frac{M_w}{M_n} \right) - 1 \right]^{0.5} M_n \tag{27}$$

Thus, the standard deviation is proportional to M_n with the square root of the incremental polydispersity exceeding unity as the proportionality constant. For a Gaussian distribution, $n_N(M)$ versus M, for example, 95% of the central area under the curve (i.e., 0.95 molar fraction of the sample) is in the range of ± 1.96 standard deviations of the peak molar mass. For any distribution shape, according to the Bienayme–Tchebycheff inequality, the molar fraction of a polymer in the range of $M_n \pm t\sigma_n$, where t is a constant exceeding unity, is at least $1 - (1/t)^2$. Thus, polydispersity alone is insufficient to define the variety of molar masses present in a polymer: M_n is also important.

Summary

The basic equations for calculating molar mass averages and molar mass distributions from both SEC and from MALDI-TOF data have been provided. The use of molar mass distributions, particularly cumulative distributions, rather than molar mass averages has been emphasized. Finally, low polydispersity does not necessarily ensure low diversity in molar masses for a sample because the value of M_n also contributes to the standard deviation of the distribution. This means that mass discrimination effects may still be serious in samples of low polydispersity if the standard deviation of the distribution is large. In the remainder of this article, we describe experimental results illustrating the use of these calculations.

EXPERIMENTAL

Materials

Narrow-distribution PMMAs were obtained from Polymer Laboratories (Amherst, MA). The broad-distribution PMMA was synthesized by free-radical addition polymerization at Eastman Kodak Co. (Rochester, NY). The matrix material, 1,8,9-anthracenetriol (dithranol), and the cationization reagent, sodium trifluoroacetate (NaTFA), were purchased from Aldrich Chemical (Milwaukee, WI) and used without further purification.

MALDI-TOF MS

Polymer samples for MALDI analysis were prepared at a concentration of 5 mg/mL in tetrahydrofuran (THF; unstabilized). The matrix solution (dithranol)

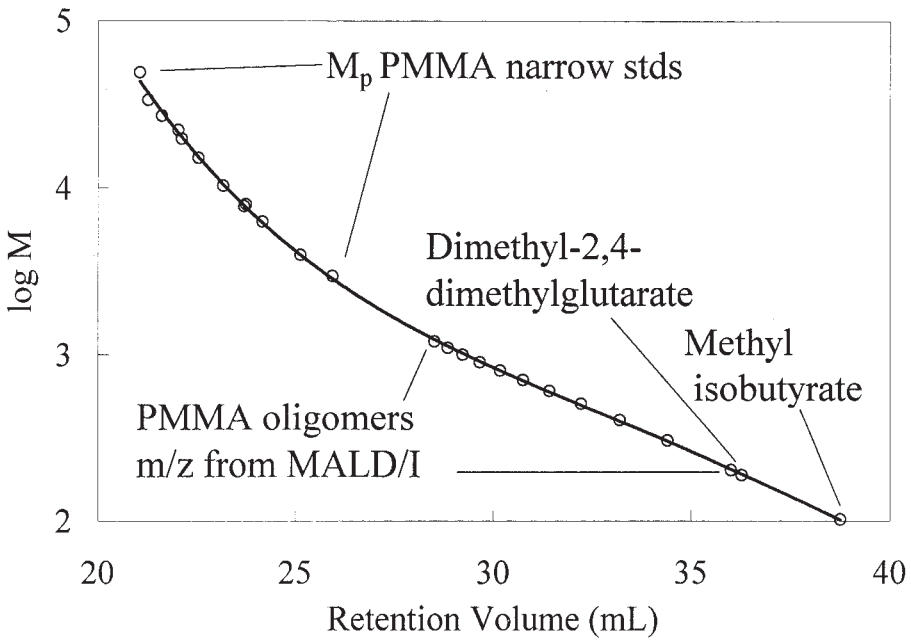

Figure 2 SEC calibration curve.

was prepared at a concentration of 28 mg/mL (0.15*M*) in THF. The cationization reagent, NaTFA, was prepared at a concentration of 1 mg/mL in THF. The samples were prepared through the mixing of the polymer solution with the dithranol solution and NaTFA solution at a volume ratio of 1:7:1. The mixture (0.5 μL) was deposited onto a sample plate and allowed to air-dry. The sample preparation conditions were not rigorously optimized. MALDI-TOF MS experiments were carried out with a TofSpec2E laser time-of-flight mass spectrometer (Micromass, Inc., Manchester, UK) equipped with dual microchannel plate detectors for both linear and reflectron modes and a nitrogen laser (337 nm). Positive ion mode was used for all analyses, with an accelerating voltage of 25 kV for linear mode and 20 kV for reflectron mode. Spectra were acquired using delayed extraction mode with a 500-ns delay time. The delayed extraction pulse voltage was optimized for resolution depending on the mass range of the individual polymer distributions.

SEC

For SEC, three 500-Å columns and one 100-Å column (7.8 × 300 mm Ultrastyragel columns) from Waters Corp. (Milford, MA) were used. The SEC system included an Agilent 1100 series isocratic pump with a multiple-wavelength UV detector and a Waters 410 DRI detector. The flow rate was nominally 1.00 mL/min and was corrected with acetone as an internal flow marker. The samples were injected in a volume of 100 μL at concentrations of 0.5 mg/mL. Dimethyl 2,4-dimethylglutarate and methyl isobutyrate were obtained from Aldrich Chemical. Narrow-distribution PMMAs were obtained from Polymer Laboratories. A narrow standard calibration curve was constructed from the M_p values supplied by the vendor for standards greater than a molar mass of 2990. The structures and masses of oligomers of less than 1202 Da were determined by MALDI-TOF MS of SEC fractions of PMMA 1035 and were used with the retention volumes of the individual oligomers to provide the calibration curve shown in Figure 2, which also includes retention volumes of dimethyl 2,4-dimethylglutarate and methyl isobutyrate. The SEC column set was selected for high efficiency, a large total column volume, and a shallow calibration curve slope to minimize axial dispersion effects. The standard deviation of a chromatogram of acetone was 0.18 mL. When this value was used to correct the experimental chromatograms by deconvolution with the method of Ishige et al,.[20] the experimental and corrected chromatograms were indistinguishable. Also, the PMMA samples of less than 10,000 Da were proton-terminated, and the variation of the specific refractive index increment, *dn/dc*, with the molar mass was small, except below a molar mass of 3000. We simplified this study by ignoring both axial dispersion and specific refractive index increment (dn/dc) corrections.

RESULTS AND DISCUSSION

Molar mass averages

The simplest comparison of MALDI-TOF MS and SEC results involves the use of molar mass averages (i.e., M_n and M_w). These comparisons begin by assuming

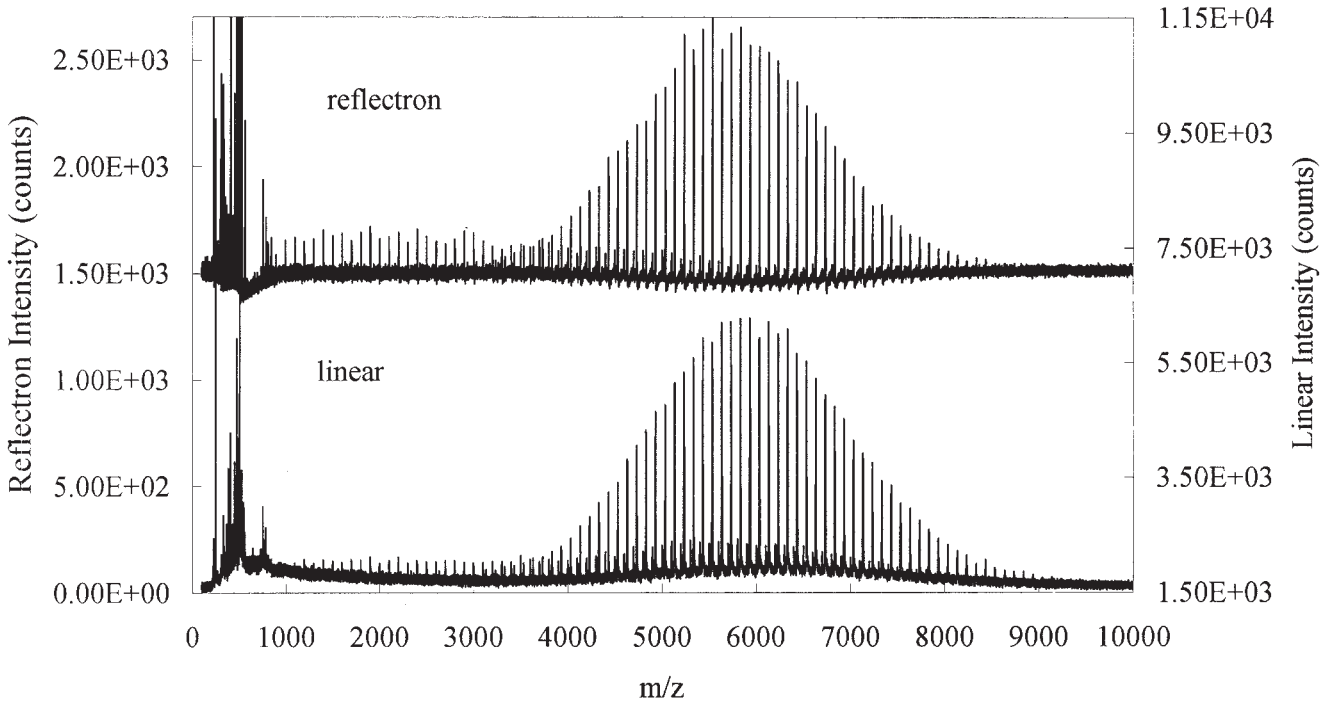

Figure 3 Linear and reflectron MALDI-TOF spectra of PMMA 6300.

that the intensity of the MALDI-TOF MS spectra is proportional to the number of molecules per molar mass increment, and the height of the SEC chromatograms is proportional to the mass of molecules per retention volume increment. For the moment, we also assume simple linear baselines for both data sets, which for MALDI-TOF MS spectra may include a positive envelope in linear mode and, in some instances, a baseline that dips to a negative value in the reflectron mode (e.g., PMMA 6300; Fig. 3). For the data presented here, the method used for setting the MALDI-TOF MS baseline was to identify the highest and lowest m/z peaks of the spectrum as the end points of a straight-line baseline, use linear regression to fit 20 points on either side of these spectral peaks, raise the baseline a distance equal to three times the standard error of the estimate (a measure of the standard deviation obtained from the regression), and, finally, set all negative heights to zero after the baseline was subtracted. A summary of the molar mass averages and polydispersities calculated from the baseline-corrected MALDI-TOF MS spectra is compared with the SEC values in Table I. One might believe that MALDI-TOF MS and SEC results for the narrow standards are in agreement, except for the highest (PMMA 22,000) and lowest (PMMA 1035) samples. Assuming that the SEC data correctly represent the distribution, we calculated the error percentage of the MALDI data for each sample. A plot of the error percentage versus the molar mass for M_n, M_w, and M_z is displayed in Figure 4. The error changes systematically from positive to negative with increasing molar mass. Also, the error percentage changes in order, progressively, from $M_z < M_w < M_n$ for PMMA 1035 to $M_n < M_w < M_z$ for PMMA 22,000.

The polydispersities of the narrow standards obtained from MALDI were consistently lower than those obtained from SEC, and this was likely due to a small amount of SEC axial dispersion. It is evident, however, that the polydispersity of the broad PMMA was grossly underestimated by MALDI-TOF MS, and all molar mass averages for the broad-distribution sample were substantially lower than the SEC values. These observations have been made for numerous polymers with broad molar mass distributions and have been attributed to mass discrimination inherent in samples with large polydispersity.[5,6,16,21,22] As mentioned previously, this has led to the suggestion that polymers with low polydispersity do not suffer from the discrimination problem. The nonrandom trends observed in the error percentage for the molar mass averages shown in Figure 4 indicate that polydispersity may not be sufficient to define whether or not MALDI mass discrimination effects will be observed because all the narrow-distribution PMMA samples had similar polydispersities. This conclusion becomes even more apparent when the molar mass distributions are examined.

Molar mass distributions

Figure 5 presents MALDI-TOF and SEC differential molar mass distributions on a molar fraction basis for

TABLE I
PMMA Molar Mass Averages from MALDI and SEC

		MALDI				SEC				Error (%)		
M_p	Mode	M_n	M_w	M_z	M_w/M_n	M_n	M_w	M_z	M_w/M_n	M_n	M_w	M_z
PMMA narrow molar mass distribution												
1,035	Linear	1,020	1,110	1,190	1.09	756	904	1,030	1.20	34.9	22.8	15.5
	Reflectron	990	1,060	1,130	1.07					31.0	17.3	9.7
2,990	Linear	2,730	2,910	3,080	1.07	2,450	2,660	2,860	1.09	11.4	9.4	7.7
	Reflectron	2,490	2,690	2,840	1.08					1.6	1.1	−0.7
4,000	Linear	3,600	3,970	4,320	1.10	3,290	3,710	4,100	1.13	9.4	7.0	5.4
	Reflectron	3,380	3,660	3,930	1.08					2.7	−1.3	−4.1
6,300	Linear	5,860	6,080	6,260	1.04	5,790	6,080	6,350	1.05	1.2	0.0	−1.4
	Reflectron	5,540	5,780	5,950	1.04					−4.3	−4.9	−6.3
10,300	Linear	8,420	9,210	9,660	1.09	8,780	9,830	10,600	1.12	−4.1	−6.3	−8.9
15,100	Linear	10,600	12,300	13,200	1.16	11,600	14,100	15,600	1.22	−8.6	−12.8	−15.4
22,200	Linear	15,800	16,200	16,700	1.03	20,600	22,400	24,300	1.09	−23.3	−27.7	−31.3
PMMA broad molar mass distribution												
	Linear	2,980	3,900	4,780	1.31	4,040	7,440	13,200	1.84	−26.2	−47.6	−63.8
	Reflectron	2,660	3,450	4,250	1.30					−34.2	−53.6	−67.8

PMMA 1035 on a linear mass scale. The MALDI-TOF MS data are plotted on a secondary y axis for visual comparison. We confirmed the identities of the smallest three oligomers (dimer, trimer, and tetramer) in the SEC distribution by fraction-collecting the individual peaks and obtaining MALDI-TOF MS spectra of the isolated oligomers. Although the three oligomers were observed with a low S/N ratio in isolated fractions by MALDI, in comparison with the SEC data, MALDI grossly underestimated the amounts of the smallest oligomers and, in fact, did not detect the dimer at all in the presence of the entire polymer distribution. The limited response of the lowest oligomers in comparison with that of the larger oligomers is thought to be due to differences in the cation binding energies.[23–25] Mass discrimination effects of low-mass PMMA oligomers are actually slightly worse than graphically depicted because, as mentioned previously, the SEC distribution has not been corrected for decreasing DRI detector response with decreasing molar mass. Cumulative distributions on the same ordinate scale (Fig. 6) are more useful for quantitative comparisons between MALDI-TOF MS and SEC. The cumulative distributions now provide further insight into the trend in the

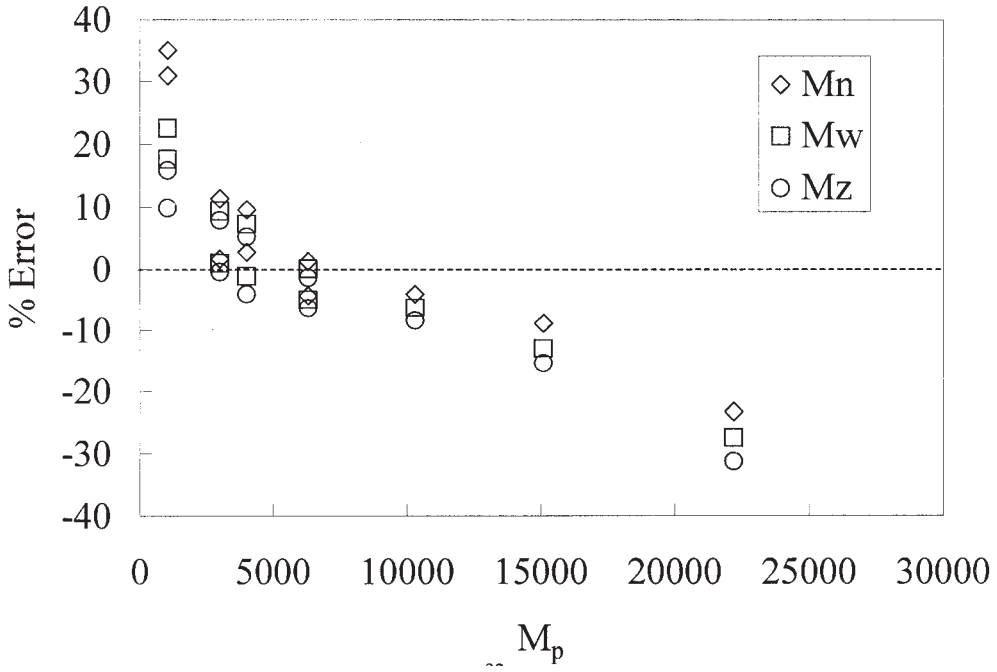

Figure 4 Error percentage for molar mass averages. The SEC values have been assumed to be correct.

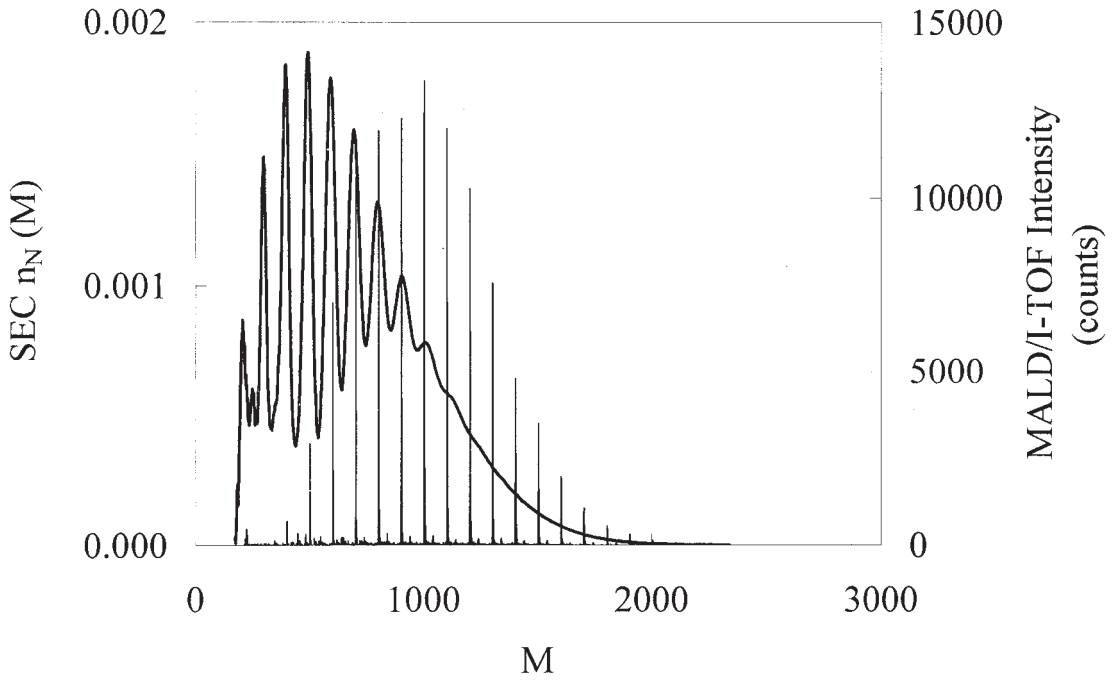

Figure 5 SEC differential molar fraction distribution and MALDI-TOF linear-mode spectrum with m/z corrected for sodium.

differences in the molar mass averages from the two techniques plotted in Figure 4. The cumulative number distributions for PMMA 1035, PMMA 2990, and PMMA 4000 are displayed in Figure 6. The SEC distributions of these three PMMA samples are displaced to a lower molar mass than the MALDI-TOF MS distributions, but they have similar shapes; this suggests a systematic error in either the SEC or MALDI-TOF distributions (or both). For now, the plots can be used to quantitate differences in the distributions but may

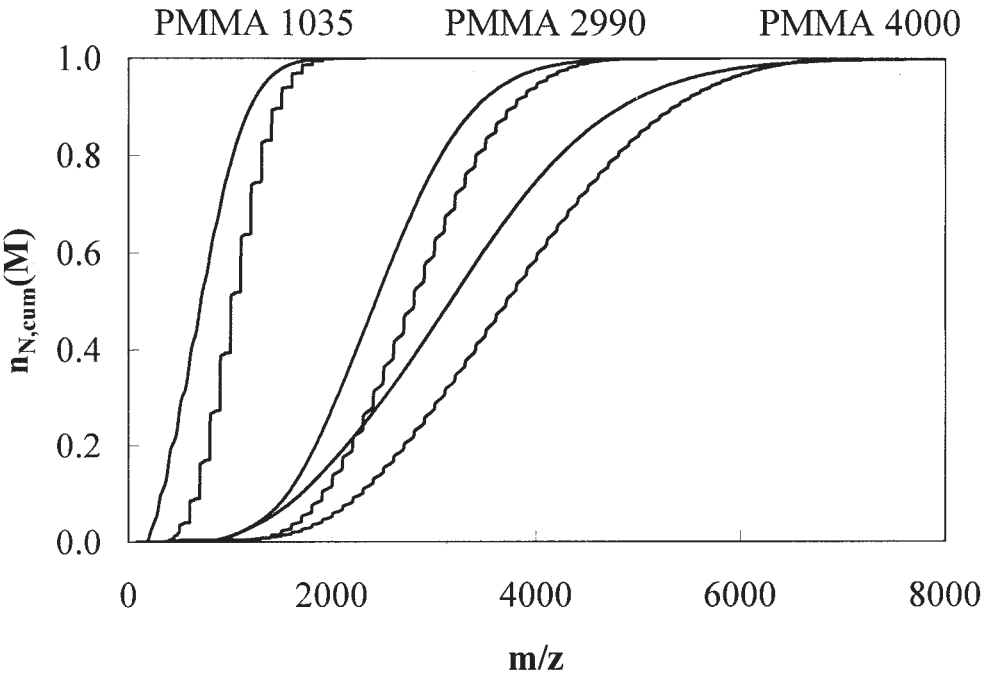

Figure 6 Cumulative number distributions of PMMA 1035, PMMA 2990, and PMMA 4000. The stepped distributions are from linear-mode MALDI-TOF MS, and the smooth distributions are from SEC.

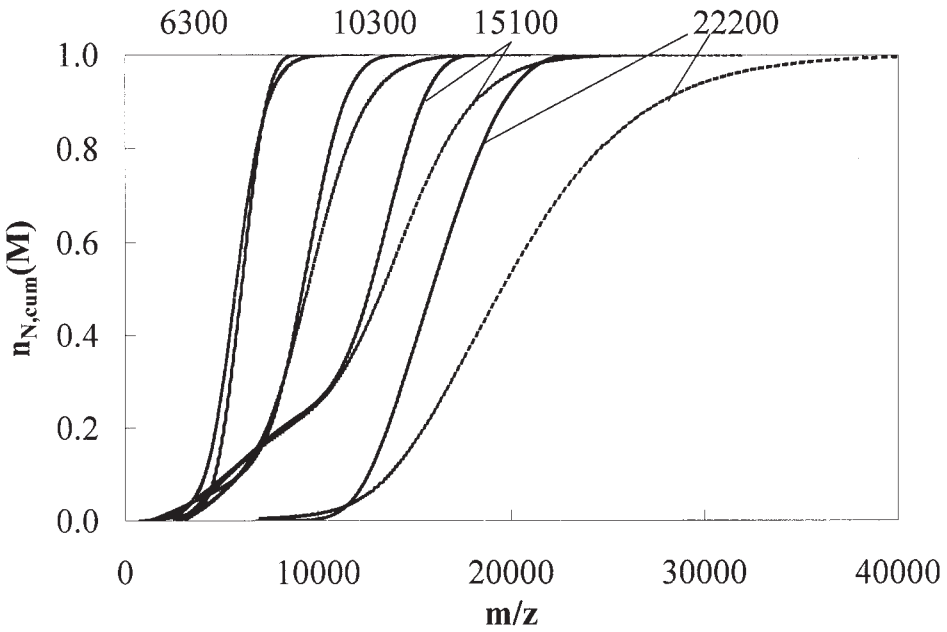

Figure 7 Cumulative number distributions of PMMA 6300, PMMA 10,300, PMMA 15,100, and PMMA 22,200. The dashed lines (lines with shallower slopes) are SEC distributions, and the solid lines are linear-mode MALDI-TOF MS.

not determine which technique(s) introduced the systematic error. One possibility is that the vendor M_p values are incorrect, and they introduced systematic error into the SEC calibration curve in Figure 2.

The cumulative number distributions for PMMA 6300, PMMA 10,300, PMMA 15,100, and PMMA 22,200 are displayed in Figure 7. The data for this set of PMMA samples indicate a different source of disagreement between SEC and MALDI-TOF MS; the shapes and breadths become increasingly different with increasing molar mass, and this indicates that the underestimation of high-mass oligomers by MALDI-TOF MS worsens as the molar mass increases. There is, of course, an overall increase in the breadth of the distributions with increasing molar mass, and this offers a more plausible explanation for the trend. Making the same comparison of the cumulative number distributions for a broad PMMA sample, as displayed in Figure 8, indicates a significant underestimation of the high-mass oligomers between distributions obtained from MALDI-TOF MS in linear mode and from SEC and an even greater discrepancy between SEC and MALDI-TOF MS in reflectron mode. When other polymer systems are analyzed by MALDI-TOF MS, comparisons of the distributions with SEC can help to guide the development of sample preparation methods for MALDI-TOF MS.

Polydispersity and mass discrimination

The previous two sections show that the MALDI-TOF MS molecular weight averages often differ significantly from those obtained by SEC, and this difference is a strong function of the peak molar mass of samples even when the samples all have the same low polydispersity (see Fig. 4). MALDI-TOF MS, in general, may benefit from data analysis that involves more than a comparison of molar mass averages. Mass discrimination effects were evident from the molar mass distributions, with very low molar masses being completely absent from the MALDI-TOF MS spectra and with high molar masses being greatly underestimated.

Table II shows the mass ranges together with the lowest and highest molar masses just visibly discernible in the MALDI-TOF MS spectra for the PMMA samples described in the previous sections. Despite the polydispersities of all the narrow samples being similar and less than 1.1, the observed mass range that the spectra span varies from approximately 2460 to 55,800. In contrast to polydispersity, as evident from Figure 9, the standard deviation obtained from eq. (27), which takes both polydispersity and M_n into account with the SEC data, correlates very well with the observed mass ranges. The observed mass range appears proportional to the standard deviation, with a proportionality constant of 8.69. If we assume that the number distribution has a Gaussian shape, a range of $\pm 4.345\sigma$ on each side of the mean (i.e., a range of 8.69σ with the mean value located at the center of the range) would encompass 99.999% of the area under the normal distribution; this is consistent with the experimentally obtained mass ranges including essentially 100% of the observed molar masses.

The limitations of the dynamic range of MALDI-TOF digitizers are among the many causes of mass discrimi-

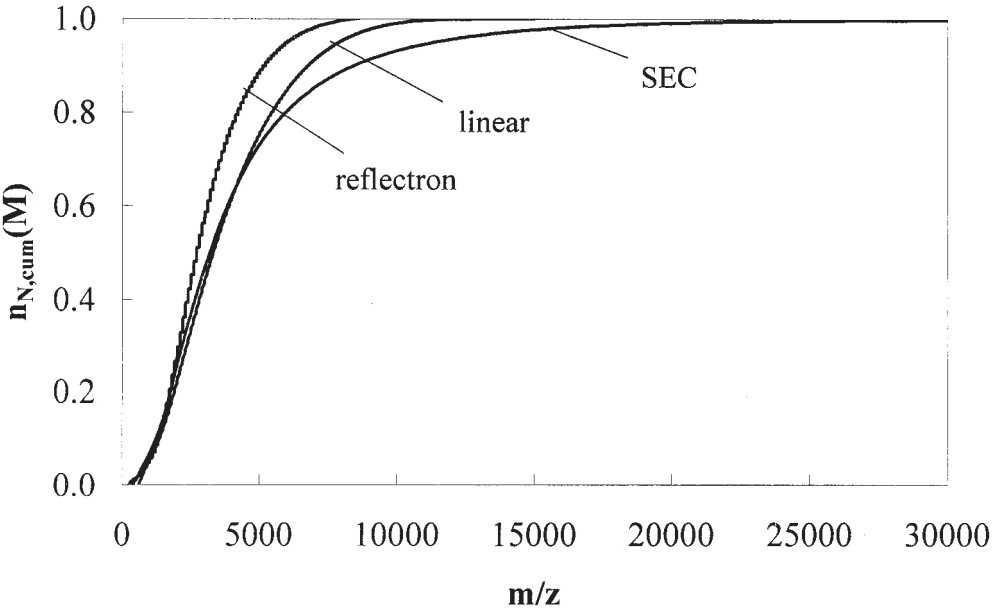

Figure 8 Cumulative number distributions for broad-distribution PMMA.

nation effects. The detection systems on most commercially available MALDI-TOF MS instruments use a 2-G sample digital oscilloscope with an 8-bit digitization capability. This allows only 255 channels of dynamic range in the *y* axis. If it is assumed that a peak needs to have an S/N ratio of 2, the usable range is roughly 2–255. This results in a dynamic range ratio of approximately 125. To show that this is generally insufficient, a measure of the required dynamic range, *R*, can be defined for a molar mass distribution [$n(M)$ vs M] as follows:

$$R = \frac{n(M)_{\max}}{n(M)_p} \tag{28}$$

where $n(M)_{\max}$ is the peak of the molar mass distribution and $n(M)_p$ is the height of the molar mass distribution at the pth quantile (e.g., if p is 0.999, then 99.9% of the moles of the polymer present are less than the molar mass selected). Taking p at a lower value will decrease R but at the expense of less accurate definition of the high-molar-mass tail of the distribution.

Table II shows values of R calculated for the molar mass distributions obtained by SEC. The values range from 1670 to 16,000 for the narrow molar mass distribution samples. For the broad PMMA molar mass distribution, the ratio is even larger: 369,000. An alternative to using a molar mass distribution obtained by SEC as a reference for the calculation of R is to use a theoretical molar mass distribution. Table III shows the results with three such distributions, all with an M_n of 5000: R ranges from 274 to 13,600. Thus, the dynamic range ratio of MALDI of approximately 125 appears to be a significant contributor to mass discrimination effects.

CONCLUSIONS

MALDI-TOF MS and SEC are, to a great extent, very powerful and complementary analytical methods for

TABLE II
Mass Ranges and Peak Intensity Estimates from SEC Molar Mass Distributions

Mp	High mass	Low mass	Mass range	$n(M)_{\max}$	$n(M)_{0.999}$	R
PMMA narrow molar mass distribution						
1,035	2,670	202	2,460	2.86×10^{-3}	3.32×10^{-7}	8,610
2,990	6,630	914	5,720	5.10×10^{-4}	6.85×10^{-8}	7,440
4,000	12,000	764	11,300	3.34×10^{-4}	5.67×10^{-8}	5,890
6,300	12,000	2,640	9,410	3.31×10^{-4}	1.98×10^{-7}	1,670
10,300	33,300	939	32,400	1.37×10^{-4}	8.54×10^{-9}	16,000
15,100	41,900	1,380	40,600	8.42×10^{-5}	1.03×10^{-8}	8,180
22,200	61,100	5,310	55,800	6.99×10^{-5}	2.50×10^{-8}	2,800
PMMA broad molar mass distribution						
	110,000	712	109,000	2.46×10^{-4}	6.67×10^{-10}	369,000

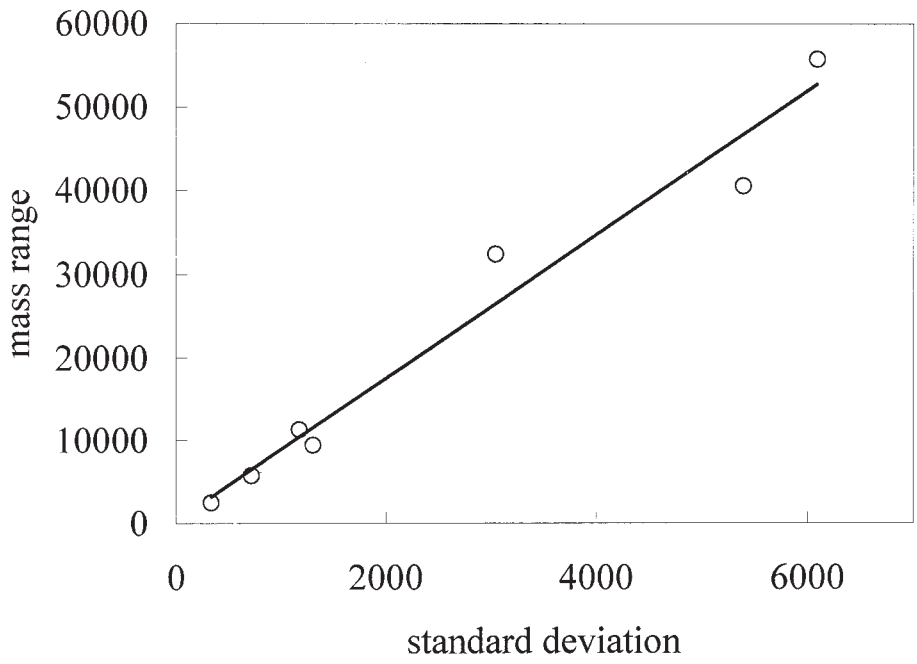

Figure 9 Mass range observed for MALDI spectra of narrow molar mass distribution PMMA standards versus the standard deviation of the molar mass distributions calculated from the SEC polydispersity and M_n.

polymers. Equations for calculating molar mass averages and molar mass distributions for both SEC and MALDI-TOF MS data have been summarized and used in an experimental analysis of PMMA. Cumulative distributions are particularly rich in information content and are easily obtained from both SEC and MALDI-TOF MS data. Mass discrimination effects in MALDI-TOF MS, which lower the concentrations of both very high and very low molar masses, combined with inaccuracies in SEC calibration curves (likely originating from inaccurate peak molar mass values in calibration standards), are suspected of contributing to differences between SEC and MALDI-TOF MS results. Molar mass ranges observed from MALDI-TOF MS data differ greatly for narrow molar mass distribution samples with the same nominal polydispersity. However, standard deviations of the distributions computed with both the polydispersity and M_n correlate very well with these observed ranges. Ratioing the extremes in molar mass concentrations measured by SEC differential refractometry, which are necessary for adequately defining molar mass distributions, has shown that values as high as approximately 370,000 are required for polydisperse samples. This demonstrates the significant limitation of current MALDI-TOF MS instrument detection systems with respect to the *y*-axis dynamic range. The prevailing opinion that MALDI spectra are representative of the polymer distribution for polymers with polydispersities less than 1.2 has been demonstrated to be not true. The limitation is not in the polydispersity but in the breadth (mass range) that the distribution covers.

TABLE III
***R* for Theoretical Distributions**

Distribution	Range of molar masses 99.9% quantile	Detector *R*
Poisson, M_n = 5,000	4,400	274
Schulz–Zimm, k = 2, M_n = 5000	28,100	2580
Flory–Schulz, M_n = 5000	47,600	13,600

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