Anal. Chem. **2000,** *72,* 4568−4576

The Limitations of MALDI-TOF Mass Spectrometry in the Analysis of Wide Polydisperse Polymers

H. C. Michelle Byrd[†] and Charles N. McEwen*[,‡]

Multisite Research in Mass Spectrometry Program: Department of Chemistry and Biochemistry, University of Delaware, 107 Brown Laboratories, Newark, Delaware 19716, and Corporate Center for Analytical Sciences, E.I. du Pont de Nemours and Company, P.O. Box 80228, Wilmington, Delaware, 19880

Average molecular weight determination of polymers with polydispersities greater than 1.2 is an ongoing challenge in the field of matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS). Mass discrimination effects observed in the analysis of these polymers have been attributed to sample preparation, desorption/ionization, and instrumental factors. In an effort to separate these factors, we studied poly(methyl methacrylate) (PMMA) standards using two different ion detection systems installed on the same time-of-flight mass analyzer. Equimass blends of narrow PMMA standards were used to simulate a polymer with a wide polydispersity. MALDI-MS analysis was also performed on a PMMA standard with a polydispersity of 1.7. All samples were analyzed by size exclusion chromatography for comparison. Although sample preparation and ionization/desorption factors were found to influence the spectral appearance of the MMA distributions, we demonstrate that, under similar sample preparation and instrument conditions, different ion detection systems produce different results for synthetic polymer blends. The differences in the detector responses for the blends and wide polydisperse standard arise from several factors related to the ion detection system: (1) detection mechanisms, (2) saturation effects, and (3) signal-to-noise limitations.

Over the past the decade, the application of mass spectrometry (MS) to polymer analysis has been influenced significantly by the implementation of matrix-assisted laser desorption/ionization (MALDI). With the introduction of this technique, it has become possible to desorb large, low-volatility polymers (up to 10^6 Da) and acquire mass spectra of the intact oligomers without the need to chemically modify polymer samples or reduce their molecular weight prior to MS analysis. From these MALDI data, structural and molecular weight information that are important for polymer characterization can readily be determined in a single fast analysis.

Various mass spectrometers are used for MALDI analysis including Fourier transform ion cyclotron resonance instruments, quadrupole ion traps and filters, electrostatic and magnetic sectors, and time-of-flight (TOF) instruments. However, the majority of commercially available instruments with MALDI capabilities are TOF mass spectrometers. TOF instruments have several characteristics that make them well suited for MALDI analysis of wide polydisperse (PD) polymers. The pulsed nature of the laser desorption makes the MALDI experiments inherently well suited for TOF instruments. Furthermore, the wide mass range capability predestines TOF MS as the instrument of choice for the analysis of synthetic polymers. Improvements in TOF technology have enhanced MALDI TOF MS to a level and speed of analysis unparalleled by any other polymer molecular weight method. This potential for direct and quick measurement of molecular weight distributions makes MALDI TOF MS especially attractive to the polymer industry.

Numerous studies on polymer molecular weight analysis have shown that MALDI-TOF MS provides reasonably accurate average molecular weight information for a variety of polymers of narrow polydispersity (PD < 1.2). However, at higher polydispersities, MALDI data are reported not to be representative of the distribution.[1−3] Some studies indicate that the high-mass components are generally underrepresented with respect to the lower mass components, resulting in significantly lower average molecular weight values.[1,2] This mass discrimination effect is thought to be caused by several factors, including sample preparation, mass-dependent desorption/ionization, ion focusing/transmission, and mass-dependent ion detection.[4−12] However, there are studies that indicate MALDI mass spectra can also show an overestimation of the high-mass components.[13,14]

* Chemistry and Biochemistry Department, University of Delaware, Newark, DE

[†] University of Delaware.

[‡] E.I. du Pont de Nemours and Co.

(1) Montaudo, G.; Montaudo, M. S.; Puglisi, C.; Samperi, F. *Rapid Commun. Mass Spectrom.* **1995**, *9*, 453−460.
(2) Montaudo, G.; Garozzo, D.; Montaudo, M. S.; Puglisi, C.; Samperi, F. *Macromolecules* **1995**, *28*, 7983−7989.
(3) Jackson, C.; Larsen, B.; McEwen C. *Anal. Chem.* **1996**, *68*, 1303−1308.
(4) Belu, A. M.; DeSimone, J. M.; Linton, R. W.; Lange, G. W.; Friedman, R. M. *J. Am. Soc. Mass Spectrom.* **1996**, *7*, 11−24.
(5) Lehrle, R. S.; Sarson, D. S. *Polym. Degrad. Stab.* **1996**, *51*, 197−204.
(6) Spickermann, J.; Martin, K.; Räder, H. J.; Müllen, K.; Schlaad, H.; Müller, A. H. E.; Krüger, R.-P. *Eur. Mass Spectrom.* **1996**, *2*, 161−165.
(7) Martin, K.; Spickermann, Räder, H. J.; Müllen, K. *Rapid Commun. Mass Spectrom.* **1996**, *10*, 1471−1474.
(8) Jackson, A. T.; Yates, H. T.; MacDonald, W. A.; Scrivens, J. H.; Critchley, G.; Brown, J.; Deery, M. J.; Jennings, K. R.; Brookes, C. *J. Am. Soc. Mass Spectrom.* **1997**, *8*, 132−139.
(9) Whittal, R. M.; Schriemer, D. C.; Li, L. *Anal. Chem.* **1997**, *69*, 2734−2741.
(10) Schriemer, D. C.; Li, L. *Anal. Chem.* **1997**, *69*, 4169−4175.
(11) Tang, X.; Dreifuss, P. A.; Vertes, A. *Rapid Commun. Mass Spectrom.* **1995**, *9*, 1141−1147.
(12) Axelsson, J.; Scrivener, E.; Haddleton, D. M.; Derrick, P. J. *Macromolecules* **1996**, *29*, 8857−8882.

10.1021/ac0002745 CCC: $19.00
Published on Web 08/31/2000

It is our experience that the sample preparation and the type of TOF instrument used in the analysis of wide polydisperse polymers has considerable influence on the appearance of the mass spectrum and the molecular weight moments measured. In an effort to separate sample preparation/desorption−ionization factors and instrumental factors, we studied a wide poly(methyl methacrylate) (PMMA) polymer and mixtures of narrow PMMA standards using two different ion detection systems installed on the same TOF mass analyzer. PMMA was selected because well-characterized standards are commercially available. Sample preparation can be standardized due to the high solubility of these polymers, as well as common polymer matrixes, in dry tetrahydrofuran. Furthermore, reliable MALDI measurements of narrow polydisperse PMMA standards over an extensive mass range have been collected on the TOF analyzer used in the present study.

EXPERIMENTAL SECTION

General Instrumentation and Conditions. MALDI experiments were carried out using a Vision 2000 time-of-flight mass spectrometer (Thermo Bioanalysis Corp.). The mass spectra were acquired in the reflectron mode operated at 5 kV with two-stage ion acceleration and 20 kV postacceleration. A nitrogen laser (337 nm, Laser Science, Inc., Newton, MA) was used with the beam attenuated to just above threshold for appearance of sodiated oligomers. Two different types of electron multipliers were used to collect the secondary electron and ion emission (as discussed below). Spectra were acquired by the summation of at least 100 laser shots selected for appearance of polymer ions. For the data shown in the present study, an external multipoint calibration was employed using poly(methyl methacrylate) polymers, the same class of polymers as that for the experiments.

The size exclusion chromatography was performed on a Waters 2690 separations module (Waters Corp., Milford, MA) using tetrahydrofuran as the mobile phase at a flow rate of 1 mL/min and an operating temperature of 30 °C. Two PLgel 5-μm Mixed C columns (Polymer Laboratories Inc., Amherst, MA) were used. The injection concentration was 1 mg/mL, and the injection volume was 100 μL. The concentration detector was a Waters 2410 refractive index detector. The molecular weight calibration for PMMA standards was created using the narrow-standards peak-position method and a PL Polymer Standards Calibration Kit with molecular weight range 1000−1 500 000 (Polymer Laboratories Inc). The Waters Millennium^{32} Chromatography Manager was used to acquire and process the data. The relative standard deviation for the weight-average molecular weight (M_w) is 3%, and 5% for the number-average molecular weight (M_n). Axial dispersion correction on the bi- and tricomponent blends was made using the Millennium routine for iterative−deconvolution.

Samples and Reagents. The narrow distribution of poly(methyl methacrylate) standards were purchased from Polymer Laboratories Inc. and the wide PMMA distribution standard (M_p = 11 700, PD = 1.7) from American Polymer Standards Corp. (Mentor, OH). These standards are referred to in the text by the manufactured designated peak-average molecular weight (M_p); e.g., PMMA 2400 is equivalent to PMMA standard having an M_p of 2400. MALDI analysis of the polymers utilized a mixture of the following compounds as organic matrixes: 2,5-dihydroxybenzoic acid (DHB) and 5-methoxy-2-hydroxybenzoic acid (MHB), both purchased from Aldrich Chemical (Milwaukee, IL), and 2,5-dihydroxybenzoic acid doped with sodium salt hydrate (DHBNa), purchased from Sigma (St. Louis, MO). The solvent, inhibitor-free tetrahydrofuran (Aldrich Chemical), stored in a Sure/Seal bottle, was used as received.

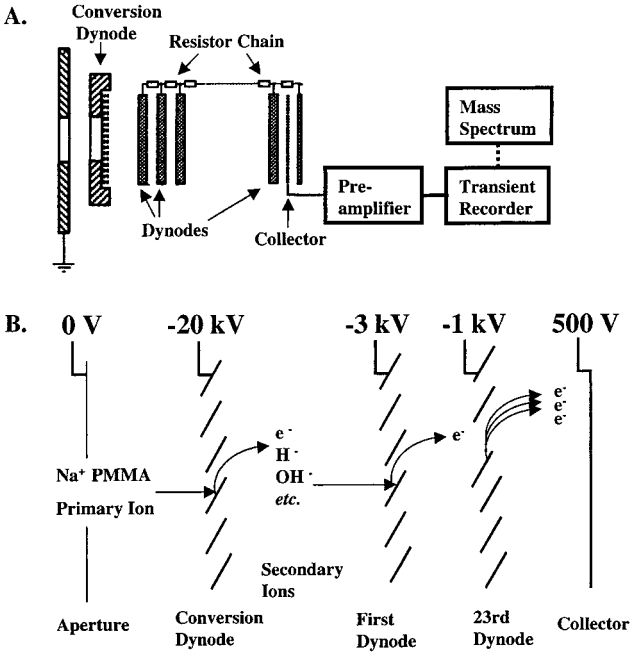

Figure 1. Schematic of (A) the discrete dynode detector and (B) the detection mechanism.

Sample Preparation. Blends were prepared by combining dry PMMA standards in the appropriate weights and then dissolving them in dry tetrahydrofuran. The manufacturer designated weight-average molecular weight (M_w) was used to determine the (average) molar concentrations. The three matrixes were combined in a ratio of (5:1:0.3) DHB/MHB/DHBNa by weight and prepared to a concentration of 1.2 M in tetrahydrofuran. Polymer and matrix solutions were stored in dry vials. In a typical MALDI experiment, the matrix and polymer solutions were premixed to give a matrix-to-analyte ratio of at least 1000:1. The premixed solutions were hand spotted onto the predried sample target and dried under N_2.

Description of Detectors. The schematics of the detectors used in this study are shown in Figures 1 and 2. Both detectors are equipped with a postacceleration/conversion dynode typically operated at −20 kV in positive ion mode, a secondary electron multiplier, and a collector. An entrance aperture, set to ground potential, serves to isolate the ion drift space from the high voltage applied to the conversion dynode.

The instrument's original detector (Figure 1A) consists of a discrete dynode electron multiplier (Hamatsu Corp., Bridgewater, NJ). The multiplier consists of 23 BeCuO dynodes. Capacitors between the 22nd and 23rd dynodes are used to reduce saturation. The detection mechanism is illustrated in Figure 1B and occurs as follows. The primary ions are accelerated into the conversion dynode producing secondary charged and neutral particles. The secondary electrons and negative ions are then accelerated into

(13) McEwen, C. N.; Jackson, C.; Larsen, B. S. *Int. J. Mass Spectrom. Ion Processes* **1997**, *160*, 387−394.
(14) Hanton, S. D.; Clark, P. A. C.; Owens, K. G. *J. Am. Soc. Mass Spectrom.* **1999**, *10*, 104−111.

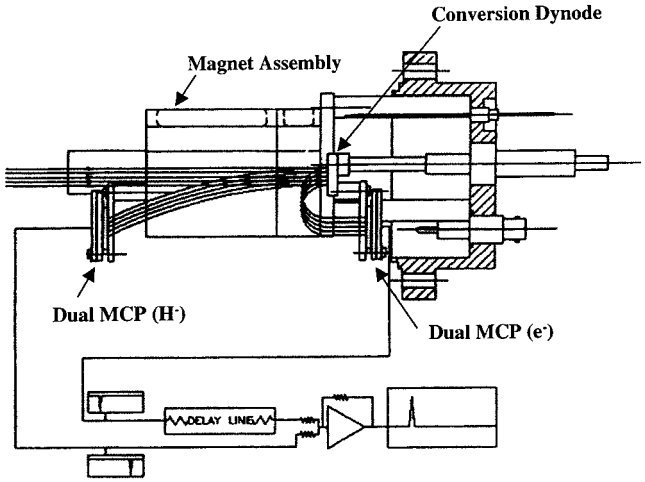

Figure 2. Schematic of the dual particle detector. A −20-kV potential was applied to the conversion dynode grid and a 2-kV potential difference across each dual MCP.

the first dynode of the electron multiplier producing multiple electrons per collision that are amplified via a collision cascading effect upon collision with subsequent dynodes. The multiplied electron beam is received at the collector, and the signal is sent to the transient recorder via an amplifier.

A disadvantage of the discrete-dynode detection system is the loss of peak resolution associated with the difference in flight times of the secondary electrons and ions in the postacceleration region.[15] This loss in resolution is circumvented by using the configuration of the dual particle detector (Scientific Analysis Instruments, Inc., Manchester, U.K.).[16] This detector (Figure 2) is equipped with a magnet. The primary ions pass through the gap of the magnet's poles and are accelerated into the conversion dynode, a solid plate composed of tantalum. Secondary electrons and anions are then accelerated normal to the dynode into the magnetic field. The magnetic field acts as a miniature mass spectrometer deflecting the particle beam as a function of mass. Due to the low magnetic field strength, only the secondary electrons and hydride ions are resolved. Separate dual-microchannel plate (MCP) detectors (Galileo Electro-Optics Corp., Galileo Park, Sturbridge, MA) receive these signals. Time-delayed circuitry is used to delay the secondary electron signal so that the secondary electron and hydride ion signals can be summed without loss of resolution.[16]

Weight Fraction to Number Fraction Conversion. In the present study, MALDI-TOF mass spectra of the PMMA blends are compared to the SEC chromatograms to illustrate the factors that may cause mass discrimination effects in the MALDI MS analysis. The mass spectral data are equivalent to the number fraction distribution, while the SEC data are related to the weight fraction distribution. To compare the SEC chromatograms to the mass spectra, SEC data must be converted to the number fraction representation. For simplicity, Figure 3 illustrates the conversion procedure in a two-step process. A typical SEC chromatogram

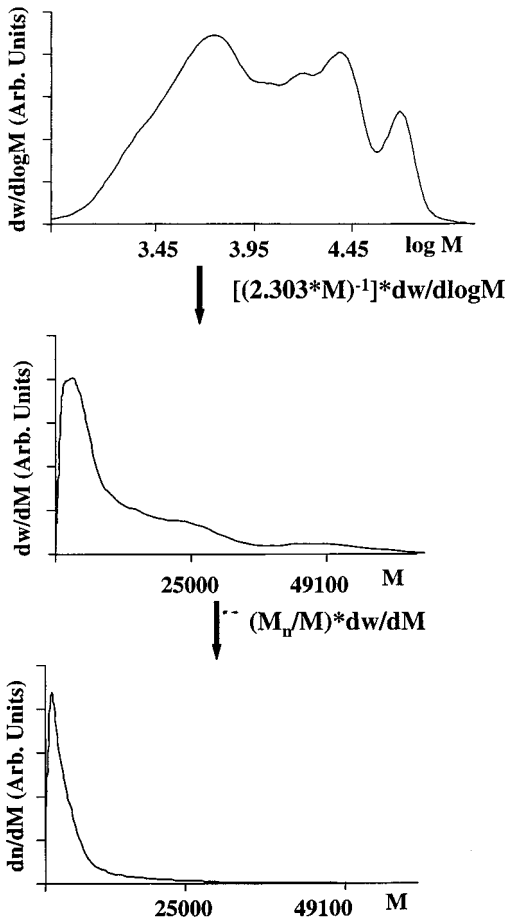

Figure 3. Conversion of a SEC weight fraction semilogarithmic plot (top) to a number fraction representation typical for mass spectrometry (bottom).

(Figure 3, top) is plotted as the differential weight fraction ($dw/d \log M$) versus $\log M$, where w represents the weight fraction and M is the molar mass. Step one converts the SEC data from the logarithmic molar mass scale to the linear mass scale (Figure 3, top and middle). To maintain the normalized area under the weight fraction distribution curve, $dw/d \log M$ must be divided by the natural logarithm of 10 and the molar mass (see equations below).

$$\text{area} = \int \frac{dw}{d \log M} d \log M = 1$$

$$d \ln M = \frac{dM}{M} \quad \text{and} \quad d \log M = \frac{d \ln M}{\ln 10}$$

$$\text{area} = \int \frac{dw}{d \log M} (\ln 10 \times M)^{-1} dM = 1$$

In step two, the differential number fraction (dn/dM) is obtained by dividing dw/dM by M and then multiplying the quotient by the number-average molecular weight M_n (Figure 3, middle and bottom). Since mass spectra presented are plotted as the signal intensity versus square root mass-to-charge ratio, an additional transformation from the linear scale to the square root scale ($dn/dM \times 2\sqrt{M}$) is required.

RESULTS AND DISCUSSION

One difficulty in determining the factors that limit the accurate molecular weight determination of wide polydisperse polymers

(15) Bahr, U.; Röhling, U.; Lautz, C.; Strupat, K.; Schürenberg, M.; Hillenkamp, F. *Int. J. Mass Spectrom. Ion Processes* **1996**, *153*, 9−21.

(16) McEwen, C. N.; Thompson, S. P.; Parr, V. C. A New Detector for Polymer Characterization by MALDI-TOF Mass Spectrometry. In *Proceedings of the 46th ASMS Conference on Mass Spectrometry and Allied Topics*, Portland, OR, May 12−16, 1996; p 1072.

(PD > 1.4) by the MALDI TOF MS technique arises from the ability to separate sample preparation, ionization/desorption, and instrumental effects. In an effort to separate these variables, PMMA standards were examined by MALDI TOF mass spectrometry using two different electron multiplier (EM) detectors under similar sample preparation and instrumental conditions. It is important to note that these measurements are a combined response for the efficiencies of ion production, transmission, and detection. In addition, these measurements reflect the matrix used, sample−matrix preparation, laser wavelength and energy, and sampling of the target in the summed scan.

Another difficulty in determining the accuracy of MALDI TOF MS analysis of wide polydisperse polymers is choosing a polymer standard representative of a wide polydisperse polymer. In the present study, equimass blends of narrow polydisperse PMMA standards covering an extensive mass range were prepared to mimic a wide polydisperse polymer. Simple bi- and tricomponent blends were selected to semiquantitatively compare the molar response of the two detectors by comparing the relative areas of the polymer distributions in the mass spectra to the expected average mole ratio. The expected average mole ratio was determined by dividing the mass of each narrow polymer distribution in the mixture by the weight-average molecular weight M_w. MALDI TOF mass spectra of more complex blends were also acquired. Molecular weight moments determined from these data were compared to those determined by size exclusion chromatography.

A further difficulty in evaluating molecular weight moments of polymers is related to our observation that different types of mass spectrometers interfaced with MALDI produce different results. Even different manufacturer's MALDI-TOF instruments produce polymer spectra for wide polydisperse polymers that are qualitatively different. It is this difference in instrumentation that accounts for some of the discrepancy in results found in the literature. For this study, spectra were acquired on a Thermo Bioanalysis Vision 2000 time-of-flight mass spectrometer operated in the reflectron mode. This instrument is a gridless system that uses low-voltage, two-stage ion acceleration. As a result, the high-mass/low-mass polymer ion ratio is about the same in the reflectron and linear modes of operation. Operating in the reflectron mode has the advantage of reducing matrix-related background by eliminating neutral particles and most fragment ions that traverse the linear sector, thus reducing detector saturation without low-mass beam deflection.

Bicomponent Blend. Equimass quantities of two narrow polydisperse standards (M_p = 2400 and 52 500) were mixed and then dissolved in tetrahydrofuran. Approximately 35 pmoles of total polymer was loaded onto the MALDI target. The analyte-to-matrix ratio was ~1:3000. SEC analysis of the blend gave signal responses consistent with an equal mass ratio. MALDI mass spectra of the bicomponent blend were acquired using the two different detectors to examine their molar response to each polymer distribution. The expected average mole ratio of PMMA 2400 to PMMA 52500 is ~21:1. The spectra shown in Figure 4 were collected using the same blend under similar sample preparation and instrumental conditions. However, the two spectra differ significantly. In the mass spectrum acquired using the discrete dynode detector (Figure 4A), the distribution of the high-mass component (M_p = 52 500) is overrepresented with respect to the low-mass component (M_p = 2400). As given in Table 1 for sample A, the peak area for PMMA 2400 is only 3 times greater than the peak area for PMMA 52500. In contrast, the data acquired with the dual particle detector show that the ratio of the peak areas for the two distributions is in closer agreement to the expected mole ratio (Table 1 and Figure 4B).

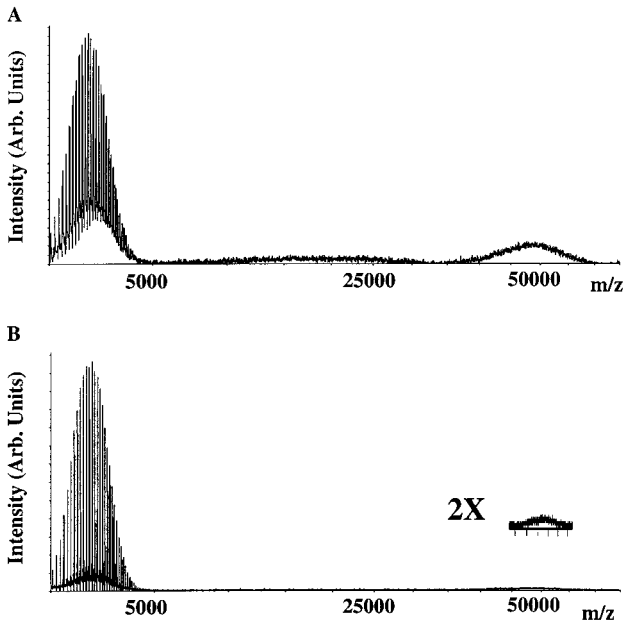

Figure 4. MALDI TOF mass spectra of an equimass bicomponent PMMA blend (M_p = 2400 and 52 500) acquired using (A) the discrete dynode electron multiplier and (B) the dual particle detector (*m*/*z* range 900−65 000). A 35-pmol sample of total polymer was loaded onto the probe in both cases.

Table 1. Ratio of Absolute Areas (*A*) of Sodiated PMMA Distributions in Equimass Bicomponent Blend (M_p = 2400 and 52 500)

	$A_{PMMA\ 2400}/A_{PMMA\ 52500}$[a]	
	sample A[b]	sample B[c]
expected	21:1	21:1
discrete dynode	4:1 ± 1 (3:1)[d]	2:1 ± 1
dual particle	15:1 ± 3 (13:1)[d]	11:1 ± 2

[a] ±*s*, standard deviation from three trials. [b] A 35-pmol sample of total polymer was loaded to MALDI target. [c] A 105-pmol sample of total polymer was loaded to MALDI target. [d] Ratio includes doubly charged PMMA 52500 and the sodiated dimer.

Close inspection of both spectra shows distributions of doubly charged ions and dimers, likely formed from MMA oligomers of PMMA 52500. This multimer formation may occur due to MMA aggregate formation in the solid matrix preparation and/or multibody gas-phase interactions.[17] The absolute areas of these ions were included in the data shown in Table 1. Their contribution slightly lowered the ratio of the two PMMA standards; however, this reduction is not statistically significant.

Interestingly, the mass spectra acquired with both detectors change with analyte concentration. The concentration of the two

(17) Danis, P. O.; Karr, D. E.; Xiong, Y.; Owens, K. G. *Rapid Commun. Mass Spectrom.* **1996**, *10*, 862−868.

PMMA standards was increased by a factor of 3 while the matrix concentration remained the same. The analyte-to-matrix ratio was ~1:1000, and 105 pmol of total polymer was loaded onto the MALDI target). Increasing the analyte concentration did not result in an increase in the observed multimer or multiply charged ion formation. Because the amounts of doubly charged and dimer products observed in the spectra were negligible, their contribution was not included in data presented in Table 1.

Tripling the analyte concentration (sample B) resulted in a decrease in the ratio of the peak areas for the two distributions (Table 1). This effect was observed for both detectors and, therefore, may not be exclusively due to an ion detection factor. A combination of sample preparation and mass-dependent desorption/ionization factors could lead to more severe overexpression of the high-mass component when the analyte concentration is increased. For example, increasing the polymer concentration in the analyte/matrix solution may alter the crystallization. Upon solvent evaporation, the sodium salt and polymer may not be evenly dispersed in the matrix. Segregation of matrix, analyte, and cationizing reagent has been reported for MALDI sample preparation of polymer samples.[14] Exclusion of the cationizing reagent from the matrix has been reported to affect the observed molecular weight distribution.[14] In the present study, the molar amount of sodium added to the analyte/matrix solution was in excess (~50:1) to the molar amount of polymer.

Another possible explanation for these results is a gas-phase competition between the matrix and the oligomers of the two PMMA standards for the sodium cation. The data shown in Table 1 indicate that MMA oligomers of the high-mass component may out-compete the low-mass oligomers for the available cations. There is evidence in the literature that, over a narrow mass range (25-mer to 50-mer), the ionization efficiency of MMA oligomers remains constant with increasing chain length.[18] However, this experiment was not performed under high sample loading conditions where gas-phase cation availability might limit equal ionization efficiency over a broad mass range.

A third explanation for the ratio of peak areas favoring the high-mass component with increasing concentration might be detector saturation caused by the matrix ions suppressing the low-mass signal. This explanation was employed for a similar result obtained by increasing the laser power.[13] Increasing the laser power would be expected to produce more matrix ions, but it is not readily apparent that increasing the sample concentration in the matrix should have the same effect.

Tricomponent Blend. MALDI TOF mass spectra of an equimass tricomponent blend of PMMA standards (M_p = 2400, 21 600, and 52 500) were also acquired using the two different detectors. Table 2 shows the expected and observed ratios of the absolute areas of the sodiated PMMA distributions in this blend. The three standards are represented in proper mole ratios for the data acquired with the dual particle detector. Similarly, for the discrete dynode detector, the peak areas of PMMA 21600 and PMMA 52500 are represented in the proper ratio (2:1). However, both higher mass components are overrepresented with respect to the lower mass PMMA 2400 distribution. Multimer formation between the higher mass components and dimerization of the high-mass component were observed in the data acquired with the discrete dynode detector. However, their contribution was minimal and not included in the Table 2. No multimer formation was observed in the data acquired with the dual particle detector, which may be attributed to its lower sensitivity to higher mass ions.

Table 2. Ratio of Absolute Areas (A) of Sodiated PMMA Distributions in Equimass Tricomponent Blend[a] (M_p = 2400, 21 600, and 52 500)

	$A_{PMMA2400}/A_{PMMA\ 21600}$[b]	$A_{PMMA\ 2400}/A_{PMMA\ 52500}$[b]	$A_{PMMA\ 21600}/A_{PMMA\ 52500}$[b]
expected	9:1	21:1	2:1
discrete dynode	1:1 ± 1	3:1 ± 1	2:1 ± 1
dual particle	8:1 ± 2	18:1 ± 2	2:1 ± 1

[a] A 38-pmol sample of total polymer was loaded to MALDI target. [b] ±s, standard deviation from three trials

The low-mass discrimination effect that is observed in the data acquired with the discrete dynode detector might be due to saturation of the EM dynodes by the secondary particle emission produced by the low-mass matrix ions at the conversion dynode. The fact that the higher mass components (PMMA 21600 and 52500) of the tricomponent blend are represented in the proper ratio suggests that the detector recovers during the flight time between ions generated from PMMA 2400 and PMMA 21600. Schriemer and Li reported similar observations for MALDI experiment of a tricomponent blend of polystyrene.[19] MALDI analysis of the polystyrene samples indicates that deflecting the matrix ions resulted in lower M_n values than not deflecting the ions.[19] This low-mass discrimination effect was attributed to detector saturation caused mainly by matrix species.[19]

The results for the bi- and tricomponent blends indicate that the dual particle detector may be less prone to this type of saturation effect due to the detector configuration. Splitting the secondary particle signals reduces the total ion current received at each MCP. Furthermore, it is well documented that low-mass ions accelerated through high potentials produce fewer secondary hydride ions than electrons upon collision with a conversion dynode.[20−26] Because of the detection configuration, each signal was monitored separately. In the spectra collected with the electron MCP detector, the matrix signal was typically 5 times more intense than the polymer ion signal but only twice as intense for the spectra collected with the hydride MCP detector. Since the low-mass matrix ions produce fewer secondary hydride ions than electrons, the hydride ion signal may cause less detector saturation than the electron signal.

(18) Larsen, B. S.; Simonsick, W. J., Jr.; McEwen, C. N. *J. Am. Soc. Mass Spectrom.* **1996**, *7*, 287−292.

(19) Schriemer, D. C.; Li, L. *Anal. Chem.* **1997**, *69*, 4176−4183.

(20) Geno, P. W.; MacFarlane, R. D. *Int. J. Mass Spectrom. Ion Processes* **1989**, *92*, 195−210.

(21) Brunelle, A.; Chaurand, S.; Della-Negra, S.; Le Beyec, Y.; Parilis, E. *Rapid Commun. Mass Spectrom.* **1997**, *11*, 353−362.

(22) Kaufmann, R.; Kirsh, D.; Rood, H.-A.; Spengler, B. *Rapid Commun. Mass Spectrom.* **1992**, *6*, 98−104.

(23) Martens, J.; Ens, W.; Standing, K. G.; Verentchikov, A. *Rapid Commun. Mass Spectrom.* **1992**, *6*, 147−157.

(24) Brunelle, A.; Chaurand, P.; Della-Negra, S.; Le Beyec, Y.; Baptista, G. B. *Int. J. Mass Spectrom. Ion Processes* **1993**, *126*, 65−73.

(25) Verentchikov, A.; Ens, W.; Martens, J.; Standing, K. G. *Int. J. Mass Spectrom. Ion Processes* **1993**, *126*, 75−83.

(26) Westmacott, G.; Ens, W.; Standing, K. G. *Nucl. Instrum. Methods* **1996**, *B108*, 282−285.

The dramatic difference in the response of the two detectors studied here may also arise from differences in their detection mechanisms. The discrete dynode detector captures the total secondary electron and ion emission (H^-, OH^-, C_2^-, etc.). In contrast, the dual particle detector collects only the secondary electron and hydride ion emission. The results from the experiments on the simple blends suggest that the summation of the secondary electron and hydride ion signal is roughly proportional to the expected primary ion signal over a broad mass range. It seems fortuitous that the gains of the two MCP detectors can be set so that the decay of the electron signal is just compensated by the rise of the hydride signal over such a wide mass range. MALDI analysis of more complex blends indicates, however, that other ion detection factors complicate the accurate measurement of wide polydisperse polymers.

Multicomponent Blends and Wide Polydisperse Standard. Multicomponent blends were examined to compare the responses of the two detectors toward mixtures that closely mimic a polymer having a polydispersity greater than 1.4. An equimass blend of nine narrow polydisperse PMMA standards (M_p = 2400, 3800, 5970, 6950, 10 300, 15 100, 21 600, 29 400, and 52 500) was prepared. The blend was premixed in a solution containing 1000 mole excess of matrix DHBsNa. One microliter of this mixture (100 pmol of total polymer) was deposited onto a target and analyzed by MALDI mass spectrometry.

Like a typical wide polydisperse polymer, the molecular weight distribution (MWD) covered by the blend is continuous over a broad mass range (1500−35 000). However, unlike typical polymers, the distribution is essentially bimodal, i.e., the mass range between 35 and 45 kDa has few polymeric species. The lull in oligomer abundance might provide a means of testing if detection differences can be observed between a continuous ion current reaching the detector as in a wide polydisperse polymer and a discontinuous ion beam as in the bi- and tricomponent blends.

Analysis of the complex blend revealed that the molecular weight moments determined from the MALDI data are sensitive to the baseline correction (see section on Signal-to-Noise Considerations). Because of this large uncertainty in the molecular weight moments determined over the full mass range of the blend, comparison of the molecular weight moments determined for the m/z range of 800−35 000 (i.e., PMMA standards, M_p = 2400−29 400) was thought to be more reliable.

Figure 5 shows the MALDI mass spectra of the 100-pmol sample acquired with the discrete dynode (Figure 5A) and dual particle (Figure 5B) detectors. The spectra show that both detectors are able to detect the high-mass component PMMA 52500. However, the observed molecular weight distributions are quite different between the detectors and different from the results expected by extrapolation from the bi- and tricomponent blends. The dramatic differences in the detector responses and the observations for the simple and complex blends arise from a combination of several factors: (1) detection mechanisms, (2) saturation effects, and (3) signal-to-noise limitations. The former factor was discussed in the previous section, and the latter two factors will be discussed in detail in the remaining sections.

Saturation Effects. The M_n and M_w values calculated from the MALDI data acquired with the discrete dynode detector are higher than those values determined by SEC analysis of the same

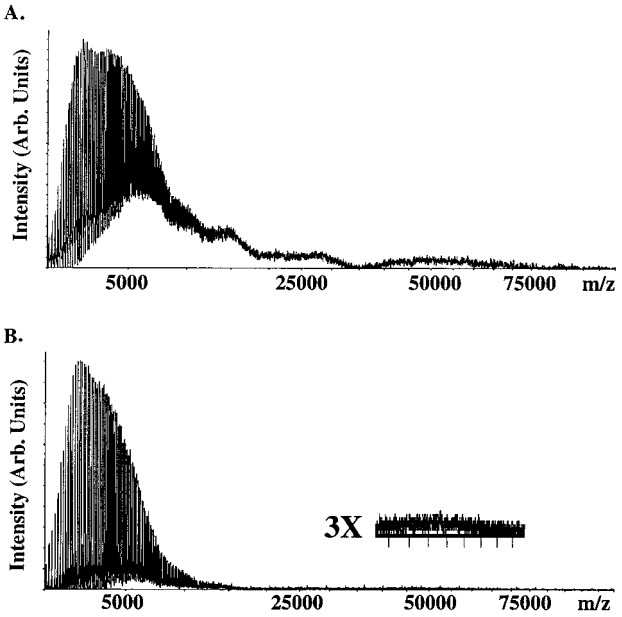

Figure 5. MALDI TOF mass spectra of an equimass multicomponent blend of nine narrow PMMA standards (M_p = 2400, 3800, 5300, 6950, 10 300, 15 100, 21 600, 29 400, and 52 500) acquired using (A) the discrete dynode electron multiplier and (B) the dual particle detector (m/z range 1000−100 000). A 100-pmol sample of total polymer was loaded onto the probe in both cases.

Table 3. Molecular Weight Moments[a] and Polydispersity (PD) Determined from MALDI and SEC Analysis of Multicomponent Equimass PMMA Blend[b] over the Mass Range 800−35 000 Da (100 pmol of Total Polymer Loaded)

MW values	MALDI data acquired with discrete dynode detector	MALDI data acquired with dual particle detector	SEC analysis
M_n[c]	7906 ± 216	5254 ± 276	5680
M_w[c]	12233 ± 334	7861 ± 241	11201
PD[c]	1.56 ± 0.05	1.50 ± 0.04	1.97

[a] Because of the large uncertainty in the molecular weight moments determined over the full mass range of the blend, comparison of the molecular weight moments determined for the m/z range 800−35 000 (i.e., PMMA 2400−29 400) was thought to be more reliable. [b] M_p = 2400, 3800, 5970, 6950, 10 300, 15 100, 21 600, 29 400, and 52 500. [c] ±s, standard deviation from three trials.

blend (Table 3). This result is not in agreement with those expected from the literature.[1,2] Comparison of the MALDI mass spectrum and the SEC number fraction representation (Figure 6A) shows overrepresentation of the oligomers in the mid-mass range (2−20 kDa) by MALDI (see Experiment Section for the conversion between SEC weight fraction and number fraction). This is not surprising since a low-mass discrimination effect was also observed in the mass spectra of the bi and tricomponent blends. This effect was attributed to saturation of the discrete dynode detector by the secondary particles produced by the matrix ions. Detector saturation may suppress the ion signal of the oligomers less than 5000 Da in the equimass multicomponent blend, resulting in a flat-topped polymer envelope (Figure 6A). As the detector recovers during the time frame of the experiment, the ion signal of the higher mass oligomers will appear to be overemphasized with respect to the lower mass ions. Figure 6B,

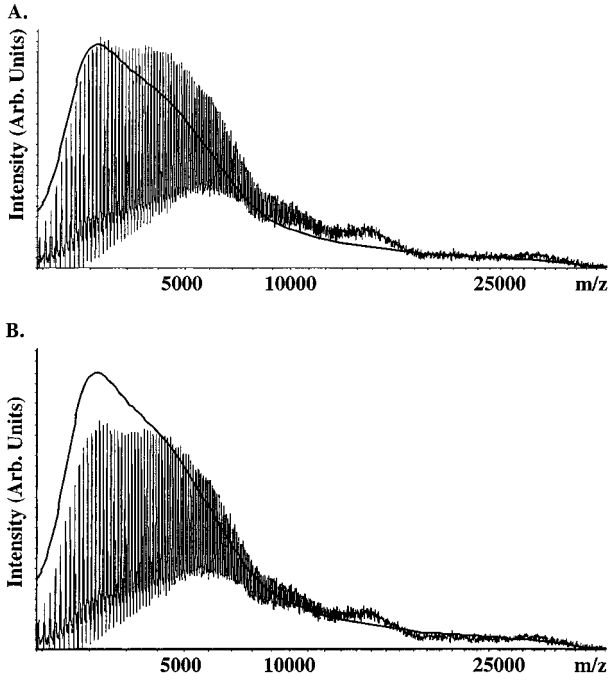

Figure 6. Comparison of SEC number fraction representation (line plot) and MALDI TOF mass spectrum of the equimass multicomponent PMMA blend acquired using the discrete dynode electron multiplier: (A) The SEC and mass spectral data are normalized to one, and (B) discrimination of low-mass MMA oligomers in MALDI mass spectrum is illustrated (*m*/*z* range 900−35 000). A 100-pmol sample of total polymer loaded to MALDI target.

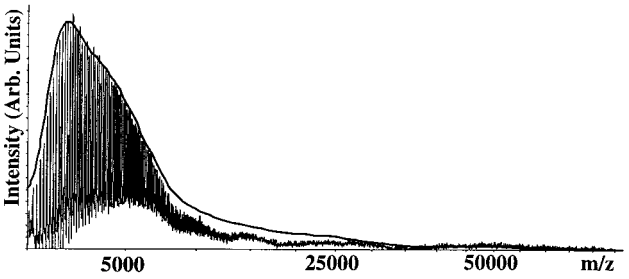

Figure 7. Comparison of SEC number fraction representation (line plot) and MALDI TOF mass spectrum of the equimass multicomponent PMMA blend acquired using the discrete dynode electron multiplier. The SEC and mass spectral data are normalized to one (*m*/*z* range 900−80 000). A 10-pmol sample of total polymer loaded to MALDI target.

which compares the SEC and mass spectrometry results with normalization to the higher mass components, seems to support suppression of the low-mass oligomer distribution.

Interestingly, by simply diluting the sample/matrix solution used above to spot 100 pmol of total polymer on the target so that only 10 pmol was loaded in the same approximate spot size, the spectral appearance for both detectors was altered in a similar way. A similar result was noted for the wide polydisperse PMMA standard. Figure 7 shows the MALDI mass spectrum of the 10-pmol equimass blend sample acquired with the discrete dynode detector. The SEC trace fits closely to the low- (<5000) and high-mass (50 000) region of the mass spectrum. The M_n value, determined over the mass range 800−35 000 Da, is comparable within experimental error to the SEC M_n value (Table 4). However, the M_w value determined from the MALDI data is significantly

Table 4. Molecular Weight Moments[a] Determined from MALDI and SEC Analysis of Multicomponent Equimass PMMA Blend[b] over the Mass Range 800−35 000 Da (10 pmol of Total Polymer Loaded)

MW values	MALDI data acquired with discrete dynode detector	SEC analysis
M_n[c]	5802 ± 252	5680
M_w[c]	9710 ± 521	11201
PD[c]	1.67 ± 0.05	1.97

[a] Because of the large uncertainty in the molecular weight moments determined over the full mass range of the blend, comparison of the molecular weight moments determined for the *m*/*z* range 800−35 000 (i.e., PMMA 2400−29 400) was thought to be more reliable. [b] M_p = 2400, 3800, 5970, 6950, 10 300, 15 100, 21 600, 29 400, and 52 500. [c] ±*s*, standard deviation from three trials.

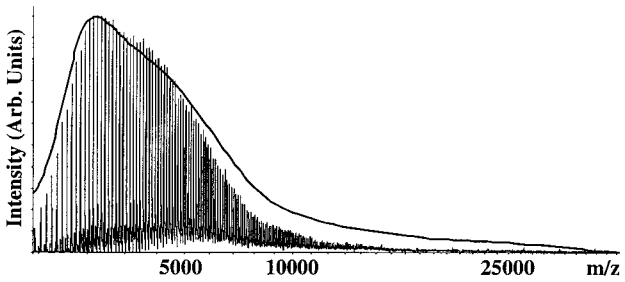

Figure 8. Discrimination of MMA oligomers in MALDI mass spectrum illustrated by comparing SEC number fraction representation (line plot) and MALDI TOF mass spectrum of the equimass multicomponent PMMA blend acquired using the dual particle detector (*m*/*z* range 900−35 000). The SEC and mass spectral data are normalized to 1. A 100-pmol sample of total polymer loaded to MALDI target.

lower than the SEC value (Table 4). Comparison of the two data sets shows underrepresentation of the oligomers between about 8 *and* 30 kDa in the mass spectrum (See Figure 7).

One explanation for these results is that, by applying a more dilute solution to the target, the resulting thin layer of matrix provides more efficient desorption/ionization of polymer analyte. If this occurs, then low-mass saturation by the matrix ions would be reduced, as appears to be the case from the appearance of the mass spectrum**.** Another explanation is related to solubility effects of the matrix and analyte in the solvent. Dilution of the original sample/matrix solution may disrupt polymer intermolecular interaction.

A second type of detector saturation may cause signal suppression in the midmass region. Detector saturation may result from continuous secondary particle emission produced by the constant flux of oligomers of this wide polydisperse blend. Detection of the PMMA 52500 distribution (Figure 7) suggests that the flight time (~50 us) between *m*/*z* 35 000 and 45 000 is sufficient for the detector to recover.

In contrast to the data acquired with the discrete dynode detector, M_n and M_w values for the MALDI data acquired using the dual particle detector are lower than the values determined from the SEC data for the equimass blend (Table 3). As compared to the SEC number fraction representation (Figure 8), the MALDI mass spectrum of the 100-pmol sample shows severe underrepresentation of sodiated oligomers greater than 6000. In the previous section, analysis of the bi- and tricomponent PMMA

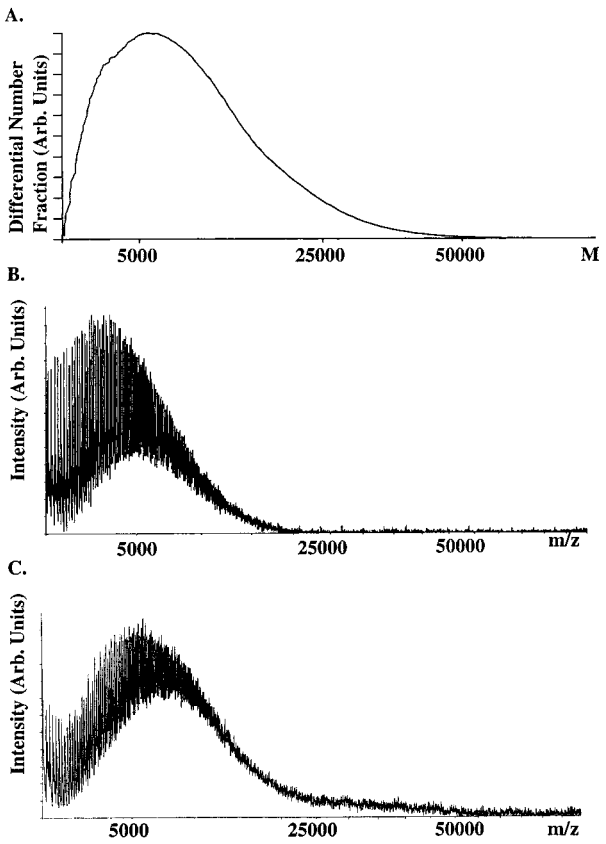

Figure 9. Wide polydisperse standard, PMMA 12 kDa, analyzed by (A) SEC (number fraction representation) and MALDI TOF MS using (B) the dual particle detector and (C) the discrete dynode detector (*m*/*z* range 900−85 000).

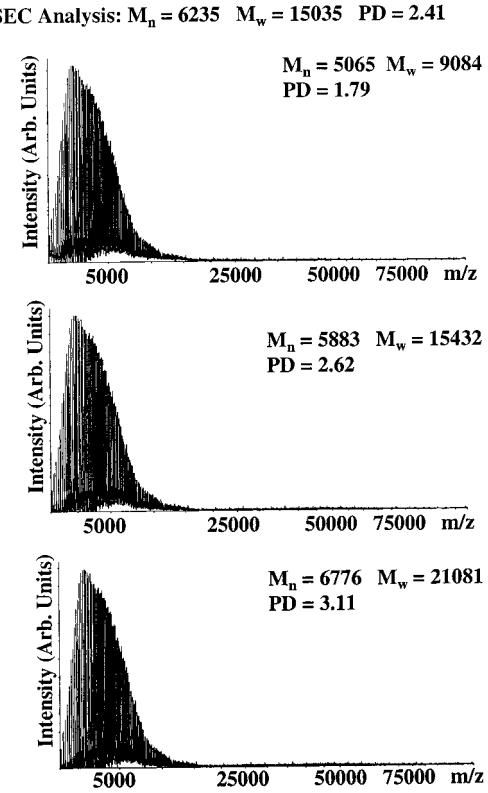

Figure 10. Sensitivity of M_n, M_w, and polydispersity values determined from MALDI TOF to baseline correction. The same MALDI mass spectral datum of equimass blend is shown using three different baseline levels (*m*/*z* range 900−80 000).

blends indicated that the dual particle detector appears to be less susceptible than the discrete dynode detector to detector saturation by the matrix ions. However, like the discrete dynode detector, the dual particle detector may suffer from saturation caused by the continuous secondary particle emission produced by the constant flux of oligomers of this wide polydisperse blend. Once again, observation of the 52 kDa component indicates that partial detector recovery is achieved. This explanation appears to be supported by the results from a PMMA 12-kDa wide polydisperse standard (Figure 9).

Figure 9 shows MALDI mass spectra and SEC number fraction representation for a wide polydisperse PMMA standard. According to the SEC analysis, the polymer distribution is continuous out to ~50 kDa (Figure 9A). Interestingly, the mass spectrum acquired with the discrete dynode detector shows ions beyond *m*/*z* 20 000, while no ions above *m*/*z* 20 000 are observed in the mass spectrum acquired with the dual particle detector (Figure 9B). Similar results were observed for the equimass multicomponent blend and were mainly attributed to detector saturation. In contrast to the wide polydisperse standard, the lull in the polymeric distribution of the complex blend allows both detectors time to recover so that the high-mass standard was observed.

Signal-to-Noise Considerations. Baseline correction is often useful when a weak analyte signal is in the presence of an intense matrix signal. Baseline correction of MALDI MS data has proven to be challenging.[27] During the course of the present study, it was observed that the average molecular weight information, determined from the mass spectral data, is sensitive to baseline correction, as illustrated in Figure 10. In this figure, MALDI data of the equimass multicomponent blend acquired with the dual particle detector are corrected using three different baseline curves. Although the three spectra appear to be similar visually, the M_n, M_w, and polydispersity values determined from the corrected data are quite different. Data analysis of these spectra indicates that a seemingly reasonable baseline function may lead to erroneous average molecular weight information. For example, no ion signal is observed between *m*/*z* 20 000 and 45 000 in the third spectrum; however, the M_w value determined from the corrected data is greater than 20 kDa. This is because the noise intensity contributes significantly to M_n and M_w determination. Since M_w is proportional to the square of the mass, the noise contribution increases dramatically with increasing mass.

The MWD of the equimass multicomponent blend mimics the distribution of a typical wide polydisperse polymer (PD > 1.4) in that the number of oligomers decreases as the mass increases. Because the number of oligomers is low at the high-mass tail, a number fraction detector produces a weak high-mass signal. Consequently, the signal-to-noise ratio (S/N) of a number-sensitive detector becomes increasingly important for the high-mass tail region of a wide polydisperse polymer (for the multicomponent blend using the dual particle detector, the S/N ratio at *m*/*z* 50 000 is ~≤3:1). The poor S/N ratio in this region not only complicates the baseline correction but also can lead to truncation of the high-

(27) Scamporrino, E.; Maravigna, P.; Vitalini, D.; Mineo, P. *Rapid Commun. Mass Spectrom.* **1998**, *12*, 646−650.

mass tail. Saucy and Zhu predicted that a significant decrease in M_w and PD for polymers with polydispersities in the range from 1.2 to 5.0 would be observed for signal-to-noise ratios less than 100:1.[28]

In contrast to number fraction detectors, weight fraction detectors, such as the differential refractometer used in SEC measurements, are sensitive to the degree of functionality in a molecule and thus provide a signal that is proportional to mass. This increased response compensates for the low number of oligomers in the high-mass tail region. Figure 3 illustrates this point by showing the relationship between the weight fraction log plot that is typical for SEC and a number fraction linear plot typical for mass spectrometry. In addition, the separation of the molecules in SEC depends on the logarithm of the molecular weight that is linear with elution volume (time). The log mass-to-volume relationship further enhances the detection of the high-mass end of the molecular weight distribution.

A similar high-mass signal enhancement occurs in time-of-flight mass spectrometry since ions of different m/z ratios are separated on a linear time scale, which translates to a square root mass scale. Unfortunately, this factor does not compensate sufficiently for the S/N limitations of the number-sensitive detectors. Even in the ideal case where these detectors yield an equal response over a broad mass range, the accurate measurement of wide polydisperse polymers would still suffer. In order for success, a high signal-to-noise ratio must be maintained over the full mass range. This S/N enhancement would facilitate the baseline correction procedure and increase the high-mass ion contribution. To gain such an improvement would require a nearly 2 order of magnitude increase in the signal intensity of the polymer ions or reduction in noise. Currently, it is unlikely that number fraction detectors combined with MALDI MS can achieve sufficient S/N enhancement without prior fractionation of the polymer.

An ion detection system that responds to the mass of the ion, as in a weight fraction detector, may potentially provide accurate molecular weight moments by MALDI mass spectrometry. Clearly, detection of all secondary negative particles produced by high-energy ion collisions with a surface gives a response that increases with mass (the discrete dynode detector used in this study), whereas detection of only secondary hydride ions and electrons gives a response that is more linear with mass (dual particle detector). This difference in the detection mechanism relates in part to the observation or lack thereof of ions greater than m/z 20 000 for the complex blend and wide polydisperse PMMA standard. One can speculate that it might be possible to create an ion detection system that incorporates both number and weight fraction detection methods. For example, increasing the primary ion impact energy at the conversion dynode could provide a means to enhance secondary ion yields in a way that is mass dependent.

(28) Saucy, D. A.; Zhu, L. Molecular Weight Polydispersity Effects in MALDI-TOF/MS and Gel Permeation Chromatography. In *Applications with Mass Spectrometry and FT-IR*; Proceedings of the 1998 Waters GPC Symposium, Phoenix, AZ, Oct 4−7, 1998; Waters Corp.: Milford, MA, 1998; pp 233−246.

CONCLUSIONS

We demonstrate that, under similar sample preparation and instrument conditions, different ion detection systems produce different results for synthetic polymer blends. For bi- and tricomponent blends, our custom-designed dual particle detector gave molar responses that were consistent with the mole ratio of the individual PMMA standards whereas our discrete dynode detector gave responses that overrepresented the high-mass oligomers. For the complex blend, the dual particle detector severely underrepresented the high-mass relative to the low-mass components, while the discrete dynode detector produced data that more closely mimic the SEC number fraction representation. These dramatic differences in the detector responses and the observations for the simple and complex blends arise from several factors related to the ion detection system: (1) detection mechanisms, (2) saturation effects, and (3) signal-to-noise limitations. Gating the matrix ion signal, adjusting the operating parameters, and working under nonsaturating conditions may minimize complications from the first two factors. However, correction of the third factor for number fraction detectors is limited by technology. Even under optimum sample preparation and instrument conditions, poor signal-to-noise ratio given by number-sensitive detectors will limit the accurate measurement of wide polydisperse polymers in MALDI-TOF mass spectrometry.

ACKNOWLEDGMENT

This work was supported by a grant from the National Science Foundation (NSF-GOALI Grant CHE 9634238). We thank Dr. R. Fuller (E.I. DuPont de Nemours & Co., Inc., Wilmington, DE) and his research team for SEC analysis of the PMMA standards. Drs. R. Fuller, C. Jackson (E.I. DuPont de Nemours), H. Barth (E.I. DuPont de Nemours), and D. Ridge (Chemistry and Biochemistry Department, University of Delaware, Newark, DE) are thanked for their intellectual contributions.

Received for review March 9, 2000. Accepted July 18, 2000.

AC0002745