Analysis of Structurally Complex Polymers by Time-Lag Focusing Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry

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ABSTRACT: The analytical utility of matrix-assisted laser desorption ionization (MALDI) mass spectrometry for the characterization of structurally complex polymers is demonstrated. The advantages and limitations of the MALDI method are discussed through the analysis of several low molecular weight copolymers. Oligomer resolution is achieved by using a MALDI time-of-flight mass spectrometer equipped with pulsed ion extraction for time-lag focusing. Accurate mass analysis of the oligomers provides data sufficient for the confirmation of the repeat unit and end group structures for alternating copolymers. It is shown that for alternating copolymers poly[(*o*-cresyl glycidyl ether)-*co*-formaldehyde] and glycidyl end-capped poly[(bisphenol A)-*co*-epichlorohydrin], the molecular weight as well as detailed structural and compositional information can be obtained from a single spectrum of each. It is found to be more difficult to obtain compositional information on block copolymers or random copolymers. Through the analysis of poly[(propylene glycol)-*b*-(ethylene glycol)-*b*-(propylene glycol)]bis(2-aminopropyl ether) and Dow polyol A and polyol B, it is shown that prior knowledge of the end group and monomer masses is required to determine information on the monomer composition. Loss of unit mass resolution complicates the analysis for monomer composition. The issue of obtaining the necessary quantitative information for complete characterization of the above polymer systems is also addressed.

Introduction

Rapid advances in polymer chemistry have generated an increasing number of new polymeric systems with very diverse composition and chemistry. A vast number of these new materials contain two or more distinct monomeric components and can be classified as block and graft copolymers, as well as alternating, gradient, and random copolymers.[1] The physical properties of these materials are understandably very dependent on type, monomer composition, and monomer sequence, in addition to molecular weight.[1−3] Determining overall chemical composition and structure is clearly important, particularly with a view to understanding structure−property relationships and the mechanisms of polymerization or copolymerization, as well as the effects of reaction conditions on polymer structure.[2,4,5]

A plethora of techniques exist that are capable of providing compositional information (average monomer content) on such polymers. A number of techniques are available for those situations where the monomeric units display different analytical composition. These include elemental analysis, functional group determination by chemical methods (e.g., titration), refractive index determination, gel permeation chromatography, pyrolysis mass spectrometry, and spectroscopic methods such as IR, UV−visible spectroscopy, and NMR.[1,6−10] If the monomeric units are chemically similar, then the cloud point titration procedure could be used to determine overall composition.[1] However, these methods cannot be universally applied as their success is dependent on the chemistry of the polymer analyzed and the sample composition. For example, discrimination between polymer blends and true copolymers can prove to be difficult, as the above techniques essentially provide bulk sample information. Therefore, it is necessary to develop analytical techniques that would provide complementary information regarding the purity and structural composition of complex polymer systems, ideally on a oligomer-by-oligomer basis.

Matrix-assisted laser desorption ionization (MALDI) mass spectrometry (MS) is a relatively new analytical tool that has a demonstrated usefulness in the mass analysis of synthetic polymers.[11−14] It is capable of providing reliable molecular weight determinations of narrow polydisperse homopolymers.[15−22] For relatively low molecular weight polymers where the instrumental resolution is sufficient to separate oligomer peaks, accurate mass analysis of the individual oligomers can be achieved, providing information on repeat unit, end group, and polymer modifications.[23,24] There are no restrictions in applying MALDI to structurally more complex polymers aside from the usual concerns of finding a suitable matrix, solvent, and cationizing reagent. However, MALDI performed in a time-of-flight (TOF) mass spectrometer has typically been lacking in the instrumental resolution necessary to provide oligomeric resolution for a wide mass range of a given polymeric system. This is particularly true as the compositional complexity increases. With the recent development of MALDI TOF systems based on the time-lag focusing principle, this mass range has been greatly extended.[25−28] For example, we have recently demonstrated oligomeric resolution for a polystyrene sample with a molecular weight of approximately 50 000.[29]

Our goal with this work is to illustrate the analytical merits of the time-lag focusing MALDI TOF system in the analysis of complex polymeric systems and to discuss its limitations. Through the analysis of several copolymers, the general utility of the MALDI technique for fast access to average molecular weight, compositional information, basic sequence information, and end group characterization is demonstrated. Some guidelines for MALDI analysis of more complex polymeric structures are provided.

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Experimental Section

Instrumentation. Mass spectral data were collected on a linear time-lag focusing MALDI TOF mass spectrometer. The basic construction of the instrument has been described elsewhere.[28] The instrument has since been modified to operate up to 30 kV for ion extraction. It features a four-plate source design with a grid inserted on the repeller side of the first extraction plate, pulsed ion extraction for time-lag focusing, and a 1-m linear flight tube. The ions are generated using the 337-nm laser beam from a nitrogen laser, having a pulse width of 3 ns (model VSL 337ND, Laser Sciences Inc., Newton, MA). A microchannel plate detector was used for ion detection (with 2 kV postacceleration), and a Hewlett-Packard MALDI data system was used for mass spectral recording and data processing. Under these experimental conditions, no metastable fragmentation could be identified in any of the polymers studied. Mass calibrations were performed externally, using well-characterized polymer standards [poly(ethylene glycol)s] analyzed in the same way as the polymers of this study. All data were reprocessed using the Igor Pro software package (WaveMetrics, Lake Oswego, OR). In general, mass spectra from 50 to 100 laser shots were summed to produce the final spectrum in this study. Number-average molecular weights (M_n) were determined from the mass domain after a correction of $1/(dm/dt)$ was applied to the data. This involves the multiplication of the intensity data by the correction factor, to account for the nonlinear correlation between the time and mass domains. Details about this correction will be published elsewhere (Schriemer and Li, to be submitted). All spectra demonstrating unit mass resolution were interpolated using 4 times zero-filling. Proton NMR spectra for the samples of poly[(bisphenol A)-*co*-epichlorohydrin] were recorded on a Bruker 400AM instrument, using 35-s relaxation delays.

Samples and Reagents. The two alternating copolymers (Aldrich Chemical Co., Milwaukee, WI) analyzed were poly[(*o*-cresyl glycidyl ether)-*co*-formaldehyde] and poly[(bisphenol A)-*co*-epichlorohydrin], glycidyl end-capped. The block copolymer poly[(propylene glycol)-*b*-(ethylene glycol)-*b*-(propylene glycol)]bis(2-aminopropyl ether) was also analyzed (Aldrich), as well as two compositionally similar proprietary copolymer polyols, Dow polyol A and polyol B (Dow Chemical Co.). All MALDI analyses utilized 2-[(4-hydroxyphenyl)azo]benzoic acid (HABA) as the organic matrix, with the exception of poly[(*o*-cresyl glycidyl ether)-*co*-formaldehyde], which required 1,4-diphenylbutadiene (Aldrich). The cationizing reagents (NaCl and $AgNO_3$) as well as the solvents (acetone and 1,4-dioxane) were reagent grade and used without further purification.

Sample Preparation. Polymer samples for MALDI analysis were prepared by combining the analyte, matrix, and cationizing agent in a common solvent. The polymer samples were dissolved in the appropriate solvent to prepare stock solutions with concentrations of approximately 5 mg/mL. With the exception of poly[(*o*-cresyl glycidyl ether)-*co*-formaldehyde], 1,4-dioxane was used as the solvent, with HABA as the matrix prepared to a concentration of 0.05 M.[24,30] Polymer stock solutions were then diluted 10-fold with the matrix solution, and 1% (v/v) of a 0.01 M NaCl aqueous solution was added, except in the analysis of glycidyl end-capped poly[(bisphenol A)-*co*-epichlorohydrin]. Acetone was used in the analysis of poly[(*o*-cresyl glycidyl ether)-*co*-formaldehyde]. The matrix 1,4-diphenylbutadiene was prepared to a concentration of 0.17 M, and the polymer stock solution was diluted 10-fold with the matrix solution. To this mixture was added 1% (v/v) of a saturated ethanolic solution of $AgNO_3$. For MALDI analysis, 1 μL of the appropriate mixture was added to the MALDI probe tip and allowed to air-dry.

Results and Discussion

The amount of information that can be extracted from a MALDI spectrum of a copolymer is potentially very great. This can be demonstrated by the analysis of two types of alternating copolymers. Figure 1 displays the MALDI mass spectrum for poly[(*o*-cresyl glycidyl ether)-*co*-formaldehyde] (PGF, polymer **1**), an alternating copolymer. The matrix 1,4-diphenylbutadiene was found suitable for the analysis, notable for its lack of acidic and/or basic functional groups, which are potentially reactive with the epoxide functional group. This polymer was analyzed as its silver adduct. Such a formulation is successful in preserving the epoxide functionality, as similar spectra were obtained from aged solutions of matrix and polymer. The expanded spectrum in Figure 1B reveals a number of features. There are three major series of peaks, labeled as P, H1, and H2, each bearing a repeat unit of 176.2 Da. The P series arises from the intact polymer **1**. The H1 series, being 56 Da lower than the P series, is likely due to the absence of one glycidyl ether group. The less intense peaks of the H2 series have masses corresponding to polymer **1** minus two glycidyl ether groups. Thus, the MALDI mass spectrum reveals that this polymer sample contains at least two other polymeric components that appear to become more dominant with increasing molecular weight. This example demonstrates that the MALDI method can provide qualitative compositional information on an oligomer-by-oligomer basis that is otherwise difficult to obtain.

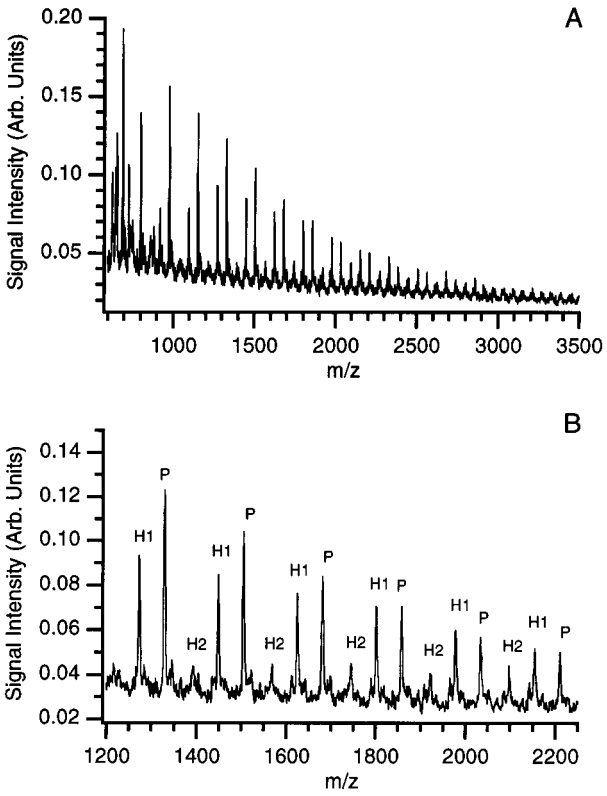
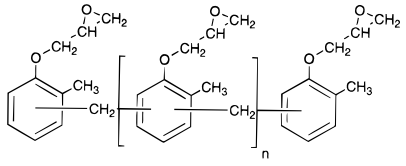


Figure 1. (A) MALDI mass spectrum of the alternating copolymer poly[(*o*-cresyl glycidyl ether)-*co*-formaldehyde] and (B) with an expansion.



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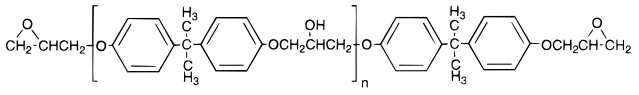
It becomes a difficult issue to determine whether or not quantitative information can be extracted from the MALDI spectra of more complex polymers. In this

context, "quantitation" implies the determination of the peak area of all oligomers in a polymer distribution and whether or not these areas accurately reflect the polymer composition. This includes whether or not average molecular weights obtained from MALDI spectra of complex polymers can be considered valid, as they are based on the relative area of the oligomers. For certain homopolymers, there is direct evidence of constant oligomer detection efficiency for those samples with a low polydispersity.[29,31,32] Indirect evidence of such efficiency for a wider class of such polymers can be found in the agreement between molecular weight data by MALDI and traditional techniques. However, the addition of structurally and chemically diverse functional groups or end groups has been known to skew the detection efficiency of the altered polymer with respect to the unaltered polymer.[23] For polymeric systems demonstrating a greater oligomer-to-oligomer compositional heterogeneity, this issue of detection efficiency likely becomes more significant. In MALDI analysis, "detection efficiency" can be used to express the combination of various factors, including efficiencies of analyte entrapment in the matrix, sample vaporization, ionization, ion transmission, and detection. These factors can have a dependency on both the mass and chemical structure of the analyte (Schriemer and Li, to be submitted). Due to the possible differences in this overall detection efficiency, the relative peak areas of different oligomer structures determined from a MALDI mass spectrum will not necessarily reflect their relative content in a polymer sample. The case involving polymer **1** represents only mild structural differences, and quantitation might be possible. The supplier has indicated an epoxide content of 0.9 for this polymer, defined as the ratio of epoxide groups to the sum of the repeat units and end groups in the polymer. The epoxide content can be determined for each oligomer in the MALDI mass spectrum. An average value of 0.94 was determined from peak areas, in good agreement with the supplier's data. This would seem to indicate that the differences in overall detection efficiency between distributions are minor in this particular case. The M_n of this polymer, considering all components, was determined to be 1417 with a polydispersity of 1.25. The supplier indicates a value of 1270 for this sample (from epoxide titration). Note that the MALDI determination of M_n is based on area calculations of peaks having masses above 600 Da. While the matrix material does not generate peaks that overlap expected oligomer peaks, none are observable below this mass.

Some very useful semiquantitative information can be obtained if a series of polymers with different molecular weight distributions are analyzed under similar experimental conditions. This can be demonstrated with the analysis of an alternating copolymer, poly[(bisphenol A)-*co*-epichlorohydrin] (PBAE, polymer **2**). Figure 2 shows the spectra of three samples of polymer **2** with nominal molecular weights of 1750, 4000, and 6100. These samples were terminated with epichlorohydrin to obtain a glycidyl end-capped product. All samples were analyzed using HABA as matrix, resulting in oligomers cationized with endogenous sodium cation.



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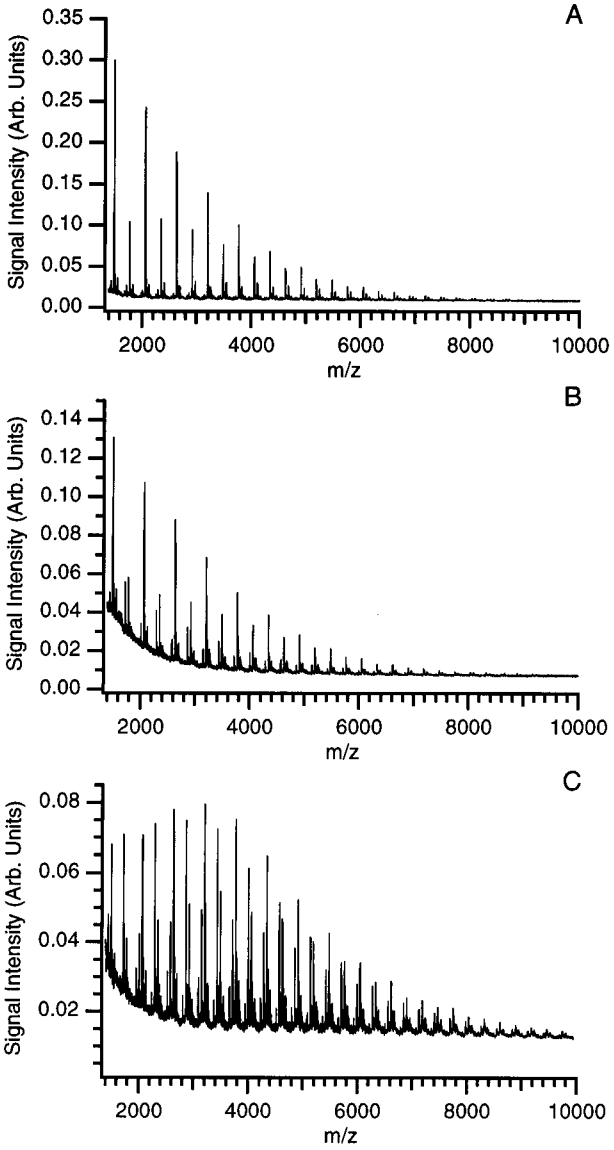

Figure 2. MALDI mass spectra of glycidyl end-capped poly[(bisphenol A)-*co*-epichlorohydrin] with nominal molecular weights of (A) 1750, (B) 4000, and (C) 6100.

The MALDI spectra shown in Figure 2 indicate that the chemical compositions of the three samples are significantly different. To examine the details of the chemical compositions, we consider two of the samples, i.e., PBAE 1750 and PBAE 6100. Portions of the expanded spectra of PBAE 1750 and PBAE 6100 are displayed in Figure 3A,B, respectively. The principal distribution labeled as P in Figure 3 has the expected repeat unit of 284.4 Da with oligomer masses that conform to the expected structure. Several other features are common to both spectra. The principal peaks in the spectra exhibit a small peak 18 Da higher (P + 18). A peak 36 Da higher than the principal peak (P + 36) is also observed. These two series of peaks likely correspond to the products from the hydrolysis of one and two epoxide end groups and appear to be present in the sample itself. Incubation of the sample in the matrix solution for variable periods of time (5 min to 2 h) does not lead to any relative increase in these peaks.

In both spectra shown in Figure 3, there are satellite peaks about the principal distribution with mass shifts equivalent to the mass of one glycidyl unit (56 Da) in the case of PBAE 1750 and 60 Da in the case of PBAE 6100 (labeled P + 56 and P + 60, respectively). In this

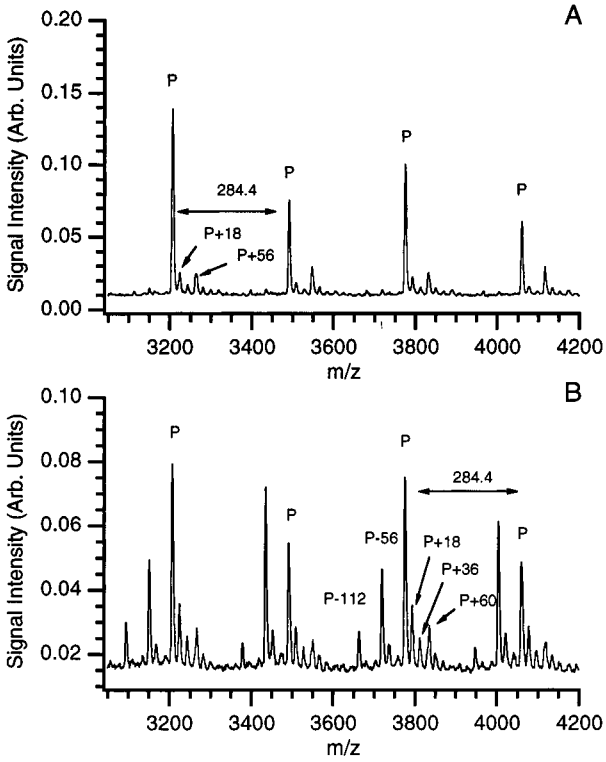

Figure 3. Expansions of the MALDI mass spectra of (A) PBAE 1750 and (B) PBAE 6100.

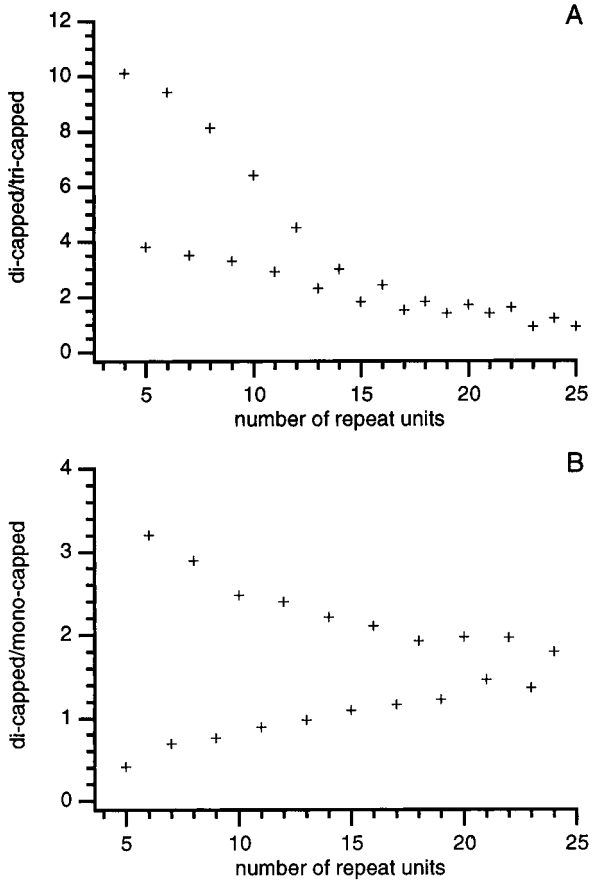

Figure 4. End group compositional analysis of (A) PBAE 1750 and (B) PBAE 6100, for the indicated oligomeric forms. Ratios are calculated from the areas of corresponding peaks.

latter case, it is clear from the spectrum that the feature with a mass shift of 60 Da partially overlaps a feature with a mass shift of 56 Da. It should be noted that the P + 60 peak begins to appear in the high-mass end of the spectrum of PBAE 1750 as well. While the identity of the peak with a mass shift of 60 Da is unknown, those with a shift of 56 Da seem to indicate the addition of a third glycidyl unit in addition to the two end caps, possibly on a secondary OH group in one of the repeat units. Furthermore, this tricapping becomes more dominant with an increase in oligomer molecular weight or chain length. Hydrolysis products of the tricapped polymer are also observed in Figure 3, as indicated by peaks +18 and +36 Da from the P + 56 series. A peak corresponding to the complete hydrolysis (+54 Da) cannot be determined with confidence due to very low signal strength. In the PBAE 1750 sample, the end capping seems to be complete; that is, there appears to be only negligible amounts of oligomers that are not terminated with two glycidyl units. The situation is different in the PBAE 6100 sample (Figure 3B). Here, end capping is obviously more distributed and incomplete. A significant portion of the sample appears to contain either one or no glycidyl end caps as indicated by the −56 and −112 Da shifts from the principal distribution (i.e., P − 56 and P − 112 peaks in Figure 3B), in addition to a fraction that is tricapped.

An interesting feature in the spectra of Figure 3 concerns the obvious intensity patterns. For example, in the principal distribution of the spectrum for PBAE 1750 (Figure 3A), the oligomers with even numbers of repeat units appear more intense than those with an odd number of repeat units. Examining the oligomer compositions of both PBAE 1750 and 6100 more closely shows that all distributions containing an even number of glycidyl units reveal this pattern. This intensity pattern is reversed for the distributions containing an odd number of glycidyl units, i.e., the oligomers with even numbers of repeat units are less intense than those with an odd number of repeat units. The variation in end capping for both PBAE 1750 and PBAE 6100 can be illustrated graphically (Figure 4A,B, respectively). For PBAE 1750, Figure 4A represents the ratio of the peak areas for the di- and triglycidyl forms of a given oligomer, showing the dependence on the number of repeat units. For PBAE 6100, Figure 4B displays a similar ratio for the di- and monoglycidyl forms of a given oligomer. The alternating patterns are clearly evident in both figures, but what is also clear is that alternating patterns tend to even out with higher molecular weight and approach a common value.

It would be enticing to derive some quantitative information on the extent of glycidyl capping from these data. However, as was expressed in the above analysis of the spectrum for poly[(*o*-cresyl glycidyl ether)-*co*-formaldehyde], one cannot tacitly assume a constant detection efficiency for all forms of the polymeric species in the absence of supporting information. In other words, although the trends and patterns of Figure 4 are quite valid, there is uncertainty as to how accurately the above ratios reflect the true oligomer content. This necessarily leads to uncertainty in the M_n values obtained from these spectra, calculated to be 3530 (D = 1.21) for PBAE 1750 and 4300 (D = 1.19) for PBAE 6100, as these calculations consider all forms of the oligomers. Perhaps a more useful approach for the comparison between samples would be to determine the number-average molecular weight of corresponding series in the spectra. For example, the M_n of the P series for PBAE 1750 is 2490 (D = 1.30) and for PBAE 6100 is 4030 (D = 1.19). If the goal of the analysis is to compare the spectrum of one product with the spectrum

of a standard or accepted product, then another option exists. Considering the present case, the product might possess oligomers with a certain ratio between mono- and diglycidyl-capping chemistry. In absolute terms, this can be expressed as follows:

$$\frac{N_{x(2)}}{N_{x(1)}} \tag{1}$$

where $N_{x(1)}$ represents the number of molecules of oligomer x with a single glycidyl unit and $N_{x(2)}$ represents the number of molecules of oligomer x with two glycidyl units. The relative areas can be obtained from the spectrum, which relates to the above ratio in the following way:

$$\frac{A_{x(2)}}{A_{x(1)}} = \frac{\sigma_{x(2)}N_{x(2)}}{\sigma_{x(1)}N_{x(1)}} \tag{2}$$

where A denotes the area of the indicated oligomer type and σ denotes the detection efficiency of the indicated oligomer type. This fraction allows one to compare oligomers in a spectrum as was done in Figure 4. As was stated previously, since the detection efficiencies of the various forms of the oligomers are not known, the area ratio does not necessarily reflect the absolute ratio as expressed in (1). However, if a given oligomer is being compared between the spectra of two samples, the following expression applies:

$$Q = \left(\frac{A_{x(2)}}{A_{x(1)}}\right)_{\mathrm{p}} \Big/ \left(\frac{A_{x(2)}}{A_{x(1)}}\right)_{\mathrm{s}} = \left(\frac{\sigma_{x(2)}N_{x(2)}}{\sigma_{x(1)}N_{x(1)}}\right)_{\mathrm{p}} \Big/ \left(\frac{\sigma_{x(2)}N_{x(2)}}{\sigma_{x(1)}N_{x(1)}}\right)_{\mathrm{s}} \tag{3}$$

where p refers to the product in question and s to the standard product. This can be reduced to the following expression:

$$\left(\frac{N_{x(2)}}{N_{x(1)}}\right)_{\mathrm{p}} = Q\left(\frac{N_{x(2)}}{N_{x(1)}}\right)_{\mathrm{s}} \tag{4}$$

where Q is obtained solely from the areas of the peaks in question. This expression removes the need for determining the detection efficiency of each oligomer, when the purpose is comparison with a standard product. The only assumption in this situation is that the detection efficiency for a given oligomer type is constant between samples, which is reasonable. Equation 4 can be applied to every oligomer in the spectra, resulting in Q values that can be plotted as a function of the number of repeat units. For illustration, such a procedure was followed for the comparison of the mono- and diglycidyl species in PBAE 4000 and PBAE 6100, where Q represents the ratio of the areas of the diglycidyl to monoglycidyl fraction between PBAE 6100 and PBAE 4000. A plot of Q versus the number of repeat units can be found in Figure 5. From the equation of the linear fit, some observations can be made. The trend in capping chemistry is virtually the same between the two samples, as indicated by a line slope close to zero and a low scatter of the points about this line. In this situation, the average Q value can be expressed by the y-intercept. Therefore, PBAE 6100 contains 0.34 times the amount of diglycidyl species contained in PBAE 4000, relative to the amount of monoglycidyl species.

Other methods for molecular weight determination only provide overall molecular weight information. For example, NMR and end group titration methods can be accurate techniques for the molecular weight determination of polymers, if the number and structure of end groups per oligomer are known with certainty.[1] The MALDI method can be used to assess the validity of the application of these techniques. As an example, a proton NMR spectrum of PBAE 1750 was obtained, from which a molecular weight of 1760 was calculated assuming all oligomers are present in the diglycidyl form. Of course, the MALDI spectrum for this polymer indicates a certain percentage of triglycidyl species, implying that the NMR-determined molecular weight is too low. Clearly, a fair comparison between the two techniques cannot be made here in the absence of information on the detection efficiency of the various forms of the oligomers in the MALDI analysis. The loss of low-mass oligomer data in the MALDI spectrum due to matrix interference is one reason why the MALDI-determined molecular weight is higher, however. As was done for the analysis of polymer **1**, a mass cutoff was established for the purpose of M_n calculation, although in the analysis of the PBAE samples this certainly neglects some of the signal for the low-mass oligomers. A cutoff of 930 Da was set for PBAE 1750 and 1450 Da for PBAE 4000 and 6100.

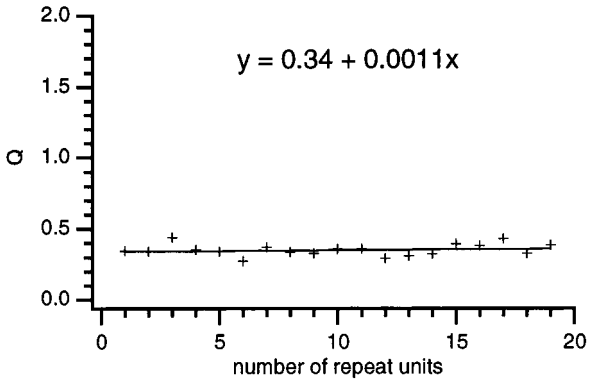

Figure 5. Comparison of PBAE 4000 and PBAE 6100 through Q, the ratio of the fraction of diglycidyl species to monoglycidyl species, over a certain mass range.

Some conclusive remarks can be made about the PBAE samples based on the MALDI data. Clearly all three samples contain more than the intended products. Hydrolysis products formed during synthesis, along with a distribution in the number of glycidyl groups, are present. The patterns evident in the spectra raise some interesting questions about the polymerization process, although such information is not available to us. Furthermore, the use of these resins as prepolymers depends on the extent of glycidyl end capping, therefore heterogeneity of capping (particularly when there is less than two glycidyl groups per oligomer) will effect the overall structure of the cross-linked polymer.[2] It is a great strength of the MALDI TOF MS technique to provide fast access to detailed structural information, allowing for rapid quality control and the ability to "fine-tune" the polymerization procedure to arrive at a more precisely engineered product.

The technique of MALDI can also provide very useful information about block copolymers or random copolymers. However, unlike alternating copolymers and homopolymers where the MALDI spectra are relatively easy to interpret by inspection due to the existence of a well-defined repeat unit, the spectra of block copolymers or random copolymers can be much more complex. For both of these types of polymers, there is no definite

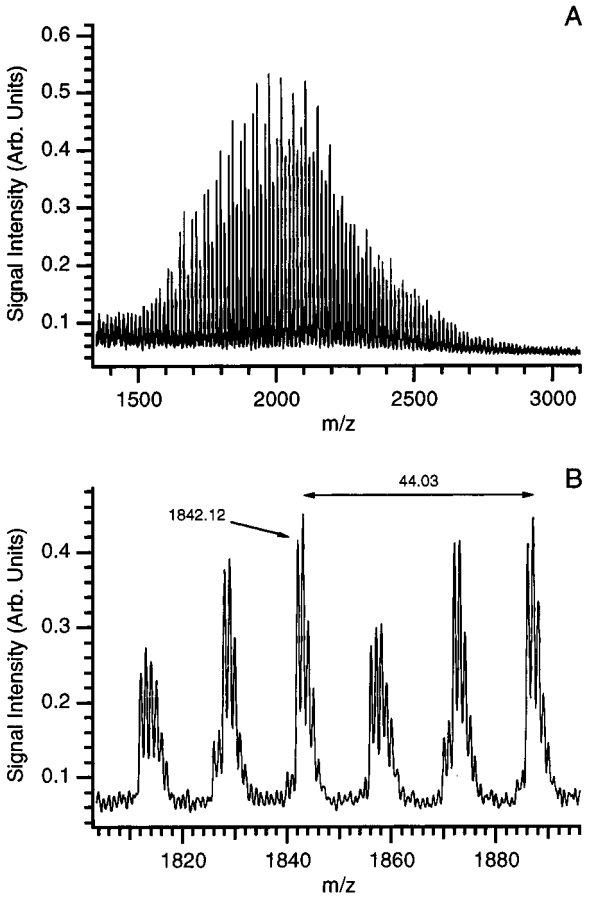

Figure 6. (A) MALDI mass spectrum of poly[(propylene glycol)-*b*-(ethylene glycol)-*b*-(propylene glycol)]bis(2-aminopropyl ether) and (B) with an expansion.

repeat unit, i.e., they contain a much higher degree of compositional heterogeneity than do alternating copolymers. This heterogeneity arises from the molecular weight as well as sequence differences from oligomer to oligomer and translates into a greater demand on instrumental resolution to extract structural and compositional information. In the spectra of homopolymers, the mass of the monomer is generally self-evident; therefore, a probable end group mass can be determined. These two pieces of information are often sufficient for establishing the overall polymeric structure. However, in the analysis of block and random copolymers, the specification of monomer and end group masses will not always be as obvious. Two examples are described below to illustrate that a great amount of compositional information can be extracted from the MALDI spectra with careful data interpretation.

Figure 6 shows the MALDI mass spectra of a low molecular weight block copolymer: poly[(propylene glycol)-*b*-(ethylene glycol)-*b*-(propylene glycol)]bis(aminopropyl ether) (polymer **3**). Our MALDI time-lag focusing instrument provides unit mass resolution across most of the displayed mass range for this polymer. It is clear from the spectrum of Figure 6B that

$$CH_3CH(NH_2)CH_2[OCH(CH_3)CH_2]_x[OCH_2CH_2]_y[OCH_2CH(CH_3)]_zNH_2$$

3

there is a pattern of three isotope clusters that repeats every 44.03 Da, from which one might postulate the presence of ethylene glycol. However, the identity of the other monomer and the end group is not directly obvious from the spectrum. To elicit compositional information from a well-resolved spectrum such as this, one requires the mass of the end group and the masses of the copolymerized monomers. Furthermore, the demands for high mass accuracy and resolution are great. If the mass accuracy is poor, there will exist more possible monomer combinations for a given mass than if the accuracy is high. In the current instrument design, a mass accuracy of better than 0.01% externally calibrated can be expected in the mass range of this polymer.

With this accuracy, a single peak representing a monoisotopic mass can be selected from one of the isotope clusters and assigned a possible monomer composition. For example, the peak with a *m/z* of 1842.12 has two possible compositions that fall within the accuracy of the mass determination, namely, 37 ethylene glycol and 2 propylene glycol monomers (calculated M + Na^+ of 1842.13 Da) or 8 ethylene glycol and 24 propylene glycol monomers (calculated M + Na^+ of 1842.29 Da). A more extensive mass range can be considered, as Table 1 demonstrates. This table shows that for a series of peaks with a mass difference corresponding to one ethylene glycol monomeric unit, one composition is favored over the other for the above-stated peak, that of 37 ethylene glycol and 2 propylene glycol monomers. The spectrum can be checked for self-consistency as a test for the validity of this assignment. The (37 ethylene glycol)/(2 propylene glycol) composition is confirmed through inspection of the rest of the distribution, as each peak in the spectrum can be assigned using this composition as a starting point. This is not true of the (8 ethylene glycol)/(24 propylene glycol) formulation, as there are peaks that cannot be assigned using this composition as the starting point. All peaks in the spectrum were then assigned relying on the combination of mass accuracy and self-consistency tests. A complete analysis of the resulting data reveals this spectrum is best viewed as a distribution of ethylene glycol oligomers (28−61 monomers in length), each with a separate distribution of propylene glycol oligomers (0−6 monomers in length). The distribution of propylene glycol monomers appears to be independent of the ethylene glycol content. Table 2 contains the peak assignments for a typical repeat pattern as is found in the spectrum of Figure 6B. These assignments identify the monoisotopic form of the indicated oligomers and provide an appreciation for the problem of block or random copolymer analysis by MALDI. Within a 30-Da mass window, five oligomers are represented. This spectral congestion would only worsen with increased compositional heterogeneity. Note that sequence information is beyond the capability of current one-dimensional MS techniques. Therefore, while it is possible to conclude that this sample is not a blend of homopolymers, it is not possible to determine if this sample is a random copolymer or a block copolymer. The assistance of NMR-based techniques or other multidimensional methods would be required to obtain this information.[8,9]

A report on the MALDI analysis of a block copolymer consisting of styrene and methylstyrene monomers has been published, whereby the average molar composition could be determined, as well as the compositional distribution of two monomer types.[4] A resolution ($m/\Delta m$) of approximately 275 was achieved for the separation of all the oligomers. However, mass accuracy was not sufficient to allow a unique assignment of the peaks,

Table 1. Comparison of Experimental Mass Data and Calculated Values for Several Peaks Shown in Figure 6

measd mass (Da)	proposed EO/PO[a]	calcd mass	mass accuracy (%)	proposed EO/PO[a]	calcd mass	mass accuracy (%)
1798.03	36/2	1798.10	0.004	7/24	1798.26	0.013
1842.12	37/2	1842.13	0.001	8/24	1842.29	0.005
1886.12	38/2	1886.15	0.002	9/24	1886.31	0.010
1930.18	39/2	1930.18	0.000	10/24	1930.34	0.008
1974.16	40/2	1974.21	0.002	11/24	1974.37	0.010
2018.11	41/2	2018.23	0.006	12/24	2018.39	0.014
2062.11	42/2	2062.26	0.008	13/24	2062.42	0.015
2106.10	43/2	2106.29	0.009	14/24	2106.45	0.016

[a] The ratio between the number of ethylene oxide repeat units and the number of propylene oxide repeat units.

Table 2. Assignment of Peaks Detected for a Typical Repeat Unit Shown in Figure 6

mead mass (Da)	EO[a]	PO[a]
1856.14	36	3
1858.11	40	0
1870.13	35	4
1872.15	39	1
1886.15	38	2

[a] EO represents the number of ethylene oxide repeat units and PO the number of propylene oxide repeat units contained in the oligomer.

requiring the aid of an NMR study. In the above analysis of polymer **3**, a resolution of approximately 2600 was achieved for isotope separation across the entire distribution, and as was noted, the mass accuracy is sufficient for unique peak identifications. This comparison underscores the importance of achieving the best possible resolution and mass accuracy.

A computer-assisted approach would be useful here, where spectral simulations are performed and fit to the experimental spectrum to obtain the relative intensities of assigned peaks. Various compositional iterations could be assessed, to arrive at the best fit to the data. Such an approach becomes essential for the extraction of relative oligomer compositions and overall average polymer composition where overlapping isotopic distributions from oligomer peaks occur. Although the isotope pattern overlap for different oligomers is identifiable (Figure 6B), the relative contributions of the different oligomers to the pattern cannot be determined directly. The computer-assisted modeling would be necessary here. Note that the determination of polymer

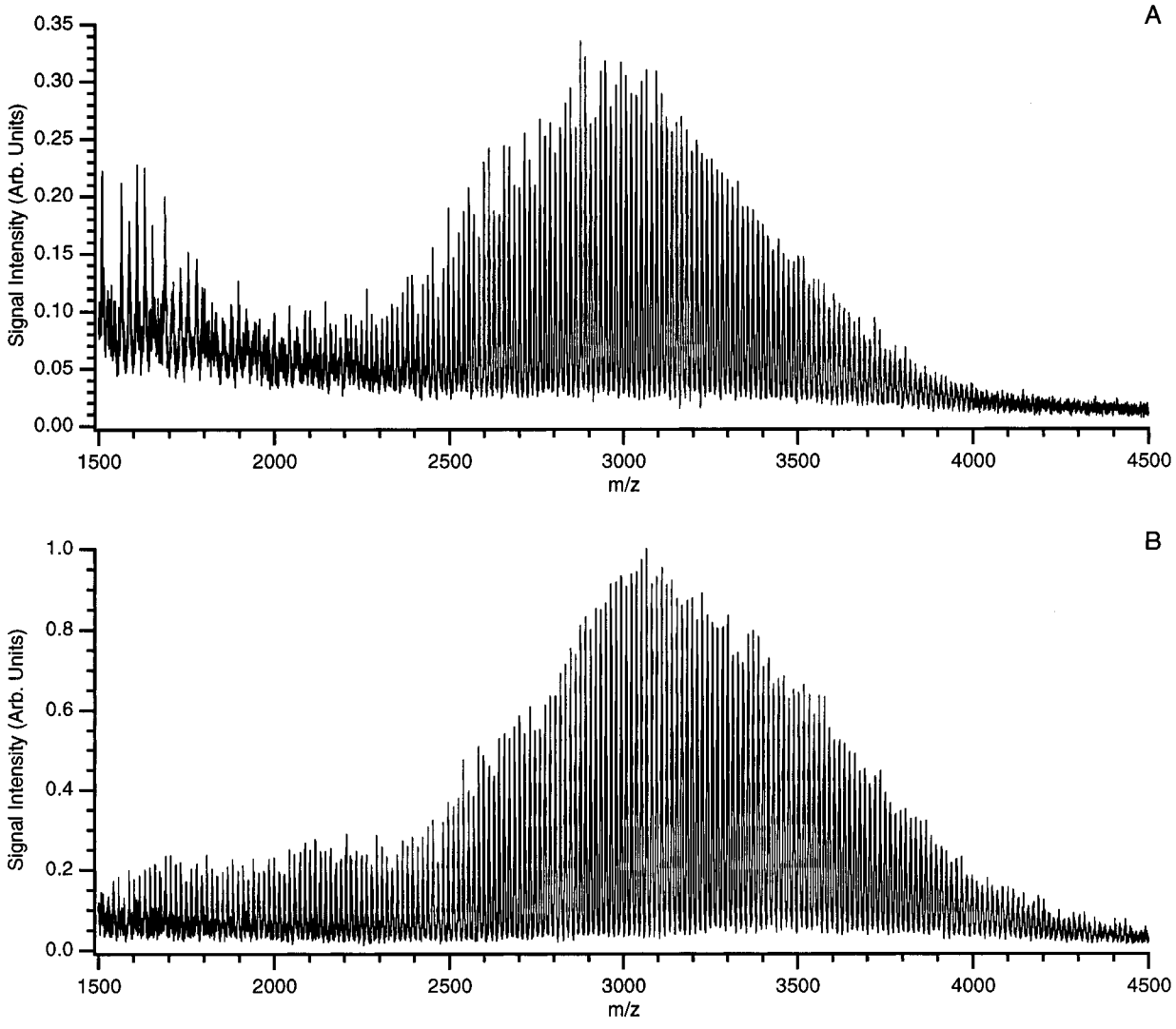

Figure 7. MALDI mass spectra of (A) Dow polyol A and (B) Dow polyol B.

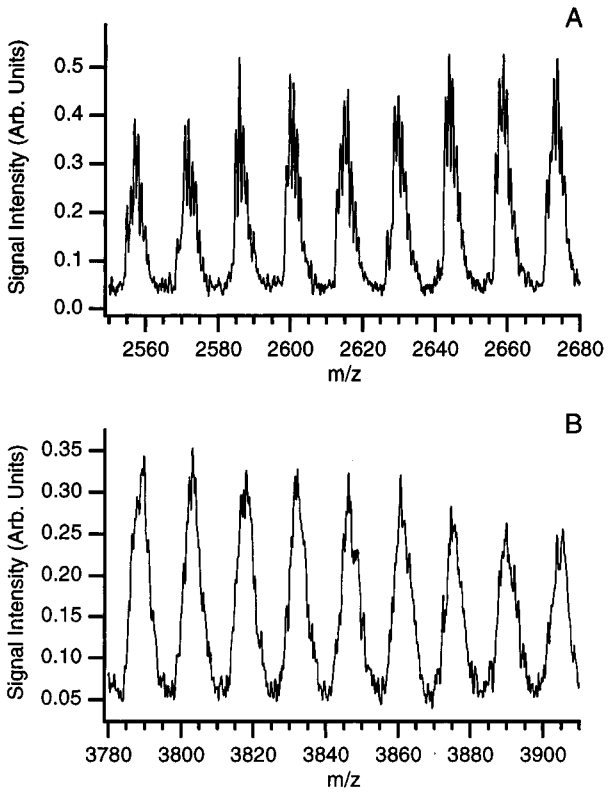

Figure 8. Expansions of the MALDI mass spectrum of Dow polyol B showing (A) the low-mass region and (B) the high-mass region.

composition by MALDI analysis also relies on the validity of extracting quantitative data across the range of composition expressed by the sample. For structurally very similar polymeric systems such as poly(ethylene glycol) and poly(propylene glycol) or polystyrene and polymethylstyrene, the assumption of uniform detection efficiency may be valid. In light of the preceding discussion on quantitation in MALDI, it is necessary to have supporting evidence of uniform detection efficiency. It is recommended to run MALDI mass spectra on individual homopolymers to assess the relative detection response. In our hands, poly(ethylene glycol) and poly(propylene glycol) homopolymers exhibit very similar analytical efficiency, leading us to assume a uniformity of response for block polymers of the two monomers. Since the computer-assisted approach is quite involved and not available to us, it is our view that the availability of such software in a commercial MALDI system would be valuable.

The situation becomes more complex with an increase in polymer molecular weight and compositional heterogeneity. This is demonstrated in Figure 7 for the analysis of ethylene glycol/propylene glycol copolymers from Dow Chemical. The expansion of the spectrum for one of the samples, Dow polyol B, is shown in Figure 8. At low mass, sufficient resolution exists to allow an analysis similar to that performed on polymer **3**. This permits a probable oligomer composition to be assigned for a well-resolved peak. However, at higher mass a number of difficulties arise to thwart an analysis by inspection. Where there is still unit mass resolution, the opportunity for determining monomer composition exists but the isotopic envelopes begin to include two *or more* possible oligomer compositions. It becomes unclear which peaks in the envelope correspond to the monoisotopic masses of the oligomers. Obviously, the left-most isotope peak in an isotope distribution corresponds to the monoisotopic peak of one (or more) oligomer in this mass range, but when *partial* oligomer overlap occurs, all the contributing monoisotopic peaks cannot be readily identified. This prevents a visual inspection of the spectrum for the determination of the monomer distribution for each oligomer, as was done for polymer **3** in Table 2. With still higher mass, the loss of unit mass resolution and the resulting drop in mass accuracy, combined with the greater number of possible oligomer compositions, prevent the determination of oligomer content from inspection.

While the MALDI method provides limited compositional information for these samples, the technique does excel at determining the effects of changes in reaction conditions. The proprietary polyols from Dow (Dow polyol A and B) are structurally similar, although prepared under different conditions. The number-average molecular weights of each were determined and found to be 3040 for Dow polyol A and 3125 for Dow polyol B, with polydispersities of 1.016 and 1.030, respectively. The mass difference is well beyond the standard deviation for such determinations (less than 1% at this mass, as determined from five separate analyses of each polymer). As for the PBAE samples, this points to the usefulness of MALDI for quality control and process monitoring.

Conclusions

It has been illustrated that low molecular weight copolymers can be analyzed by using MALDI mass spectrometry. The time-lag focusing MALDI instrument can provide improved mass resolution and mass measurement accuracy, facilitating copolymer characterization. Detailed structural information can be obtained from a copolymer mass spectrum, providing that repeat unit and end group masses are known or can be determined. In the absence of an identifiable repeat unit and end group that is characteristic of copolymer structure, the information content of a mass spectrum is reduced. This is the case for the block copolymer systems studied. If some supporting information or prior knowledge of the end group mass and monomer mass is known, compositional information can still be extracted from the MALDI spectra of block copolymers. The amount of information attainable is conditional upon resolution and mass accuracy. When both are sufficiently high, oligomer compositions can be determined with the possibility of determining an overall average monomer composition. With an increase in mass, one experiences a loss in mass accuracy and resolution such that detailed compositional analysis becomes very difficult. Achieving *sufficient* mass accuracy and resolution is the important issue here. The structural complexity of a polymeric system dictates the required resolution and mass accuracy. The recently developed time-lag focusing TOF instrumentation widens the applicability of MALDI for polymer analysis. However, the MALDI method can still reveal subtle molecular weight differences in situations where suitable resolution and mass accuracy are not present, as illustrated from the analysis of Dow polyol A and B.

The accuracy of the data based on relative peak area determinations (quantitation) will only be as good as the assumption of uniform detection efficiency across the range of compositions being investigated. This assumption should ideally be validated by comparing data with complementary techniques or through the analysis of well-characterized samples such as the corresponding homopolymer systems in the study of a

block copolymer. Clearly, more information from the PBAE samples, for example, could be obtained if the overall detection efficiencies of the various components were known. However, if the goal of the analysis is to compare the spectrum of a product with that from a standard reference material, then a more quantitative procedure can be followed.

Rapid access to detailed structural information gained from the MALDI analysis of structurally complex polymers is useful for defining chemical properties, monitoring the progress of reactions, and fine-tuning a polymerization procedure to arrive at a more precisely engineered product. Finally, the general applicability of the MALDI method for other copolymers besides those shown here is dependent on the existence of a proper matrix/sample preparation protocol.

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