Macromolecules **1995,** *28,* 1229–1232

Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry Characterization of Poly(butyl methacrylate) Synthesized by Group-Transfer Polymerization

P. O. Danis,*,† D. E. Karr,*,† W. J. Simonsick, Jr.,‡ and D. T. Wu‡

Research Laboratories, Rohm and Haas Company, 727 Norristown Road,
Spring House, Pennsylvania 19477, and Marshall R&D Laboratory, E.I. duPont de
Nemours and Company, 3500 Grays Ferry Avenue, Philadelphia, Pennsylvania 19146

Received May 11, 1994; Revised Manuscript Received October 25, 1994®

ABSTRACT: Matrix-assisted laser desorption/ionization time-of-flight (MALDI/TOF) mass spectrometry has been used for the analysis of poly(butyl methacrylate) synthesized by group-transfer polymerization, and the absolute molecular weight distributions have been determined for polymers up to 100 000. For lower molecular weight poly(butyl methacrylate) we obtained structural information confirming composition and end groups. The compounds were desorbed from *trans*-3-indoleacrylic acid and detected as positive ions showing molecular weight and several multiply charged distributions from submilligram amounts of sample. The results provided answers to ambiguities which two separate GPC measurements had created. MALDI results were supported by light scattering measurements.

Introduction

The relatively new technique of matrix-assisted laser desorption/ionization (MALDI) time-of-flight mass spectrometry is established as a powerful method for the determination of molecular weight for biopolymers such as proteins,[1-6] oligonucleotides,[7-9] and polysaccharides,[10,11] and recent results[12-20] have also shown its utility as a polymer molecular weight tool. MALDI has no reliance on polymer standards or Mark–Houwink constants for molecular weight assignments. The distribution from which the weight averages can be computed is obtained in less than 15 min from submilligram amounts of material, and no polymer molecular weight calibrants are necessary. Absolute molecular weights (as opposed to the relative values often reported) on narrow distribution polymers have been determined with an accuracy as good as or better than light scattering, osmometry, and viscometry,[14,15] and polymer distributions and polydispersity indices from MALDI agree well with those from GPC.[13-15,18] Previous work has shown that polymers of up to 400 000 can be measured with this technique.[20]

We applied MALDI to provide absolute molecular weight distributions of poly(butyl methacrylate) up to 100 000 which were synthesized by group-transfer polymerization. Group-transfer polymerization (GTP) is a relatively new polymerization technique which produces living methacrylate polymers with narrow molecular weight distribution.[21] The degree of polymerization is controlled by the monomer/initiator ratio.[21] We selected to study butyl methacrylate polymers synthesized by GTP because of their narrow polydispersities and their well-known chemistry. The narrow polydispersity ($d < 1.1$) results in an increase in the MALDI signal because the ion current is divided over a small mass range as compared to polydisperse polymers. The initiation, chain-growth, and termination reactions are understood in GTP; therefore, the end groups are known. These polymers could be used as standard materials if accurate information on their weights and composition could be obtained. GPC can provide M_w information, but it is not absolute, and results can vary widely from one lab to another. In fact, two separate GPC analyses which were performed in different facilities under different conditions for these materials provided M_w assignments which were very different (50%) from one another, but they did confirm narrow distributions ($d < 1.2$) for the materials. We report here the use of MALDI for absolute molecular weight measurements of poly(butyl methacrylate). In addition, MALDI/TOF is used to confirm composition and end groups for lower molecular weight polymers where the instrument is able to resolve the individual oligomers. For additional support of MALDI as a polymer characterization tool, we obtained light scattering results on the higher molecular weight polymers and NMR spectroscopy on the lower molecular weight polymers.

Experimental Section

Measurements were performed using a Bruker (Billerica, MA) REFLEX MALDI/TOF mass spectrometer. This time-of-flight instrument is fitted with a reflectron and dual microchannel plate detector for high-resolution analysis and with a three-stage detector in the linear mode for lower resolution molecular weight distribution measurements. The linear detector consists of a discrete dynode followed by a single microchannel plate. Following the microchannel plate there is variable acceleration (0–20 kV; 12 kV was used for these experiments) of the electrons into a scintillator and the resulting photons are detected with a conventional photomultiplier tube. The resolution ($m/\Delta m$ at fwhm) of the linear detector is about 300 for typical operations, while in the reflector mode the resolution is approximately 800. Measurements shown were made in the reflector mode or the linear mode with the three-stage detector. The matrix used for all experiments was *trans*-3-indoleacrylic acid (Aldrich, Milwaukee, WI). Samples were prepared by dissolving the polymer in acetone at a concentration of 1×10^{-4} M. A total of 4 μL of this solution was added to 20 μL of a 0.2 M solution of matrix also dissolved in acetone. This final solution was shaken briefly, and 0.5–2 μL was applied to a stainless steel probe tip with a Wiretrol II glass capillary pipet (Drummond Scientific, Broomall, PA), resulting in a total loading of polymer of 40 ng to 4 μg depending on polymer molecular weight. Ions were formed by laser desorption at 337 nm (LSI N_2 laser, 3 ns pulse width, 10^6–10^7 W/cm^2, 100 μm diameter spot), accelerated to 30 kV, and detected as the positive ions in all cases.

---

* Corresponding author.

† Rohm and Haas Co.

‡ E.I. duPont de Nemours and Co.

® Abstract published in *Advance ACS Abstracts,* January 1, 1995.

0024-9297/95/2228-1229$09.00/0

The spectra are the sum of 100 laser shots acquired at a 5-Hz repetition rate for a total acquisition time of 20 s/spectrum. The total analysis time including sample preparation was less than 15 min/sample. An external calibration using bovine insulin and angiotension II (Sigma, St. Louis, MO) was used; this provides mass accuracy within 0.02% for this instrument. Molecular weights were calculated using POLYMASS software provided by Bruker and operated on a Sun Sparcstation LX. All spectra were smoothed with a 5 or 11 point Golay–Sawitski smoothing algorithm.

Six samples of poly(butyl methacrylate) (approximate M_w 3000–100 000) were prepared by group-transfer polymerization[22] using a tetra-*n*-butylammonium biacetate catalyst (0.11 mol % relative to initiator). The initiator used for all polymerizations was 1-butoxy-1-(trimethylsiloxy)-2-methyl-1-propene.[23] This initiator produces butyl methacrylate chains with saturated butyl methacrylate end groups.

GPC 1 analyses were carried out using a Hewlett-Packard 1090 liquid chromatograph system equipped with a Model 401 differential refractive index detector (Palo Alto, CA). A four-column set, consisting of 10^5, 10^4, 10^3, and 10^2 Å μ-Styragel columns (Waters), was employed. A tetrahydrofuran (THF) mobile phase at 35 °C with a flow rate of 2 mL/min was used. External polystyrene calibrants were used in all cases. GPC 2 analyses were conducted on a Waters Model 244 GPC (Milford, MA) using a THF mobile phase. A Hewlett-Packard Model R 401 differential refractive index detector was used in GPC 2 studies. A four-column set, consisting of 10^6, 10^5, 10^4, and 500 Å Ultrastyragel columns, was used. A flow rate of 1 mL/min at a temperature of 25 °C was used. A set of polystyrene calibrants different from those used in GPC 1 was employed. Using different operating parameters and a different set of calibrants can lead to variation in the determination of molecular weights, and this situation is not uncommon, as many laboratories employ various operating parameters to obtain their molecular weight information.

Low-angle laser light scattering (LALLS) data were obtained from a GPC coupled to a light scattering detector. The GPC employed a Waters M6000 pump with a Waters Utrastyragel mixed linear column containing an average particle size of 7 μm. A THF mobile phase with a flow rate of 1 mL/min was used; the analyses were at ambient temperature. The light scattering detector was a Chromatix KMX 6 LALLS detector (Riviera Beach, FL) using a HeNe laser at 633 nm. The scattering angle was 6–7°, the field stop aperture was 0.15 mm, and the incident light was at G0 = 1000 units. Data were processed using in-house processing software.

NMR data were acquired on a Bruker AMX500 spectrometer (^{1}H NMR, 500 MHz). The ^{1}H NMR spectrum was acquired on an inverse detection probe using approximately 5 mg of polymer in 0.8 mL of $CDCl_3$ (Cambridge Isotope Labs, Boston, MA). Parameters included an excitation pulse of 3 μs (~30%), an acquisition time of 2.0 s, a relaxation time delay of 1.0 s, a sweep width of 4032 Hz, a 16K complex data set, and 3072 transients. The spectrum was Fourier transformed without apodization. The ^{13}C NMR spectrum and the ^{13}C DEPT spectrum were acquired on a 10-mm broad-band probe tuned to the ^{13}C frequency (125.77 MHz) using approximately 250 mg of polymer in 2.0 mL of $CDCl_3$. For the ^{13}C NMR spectrum, an excitation pulse of 10 μs (~60%) was used. The ^{13}C DEPT spectrum was acquired using the standard pulse sequence[24] with a 135° read pulse. Parameters for both spectra included an acquisition time of 0.6 s, a relaxation delay of 0.5 s, a sweep width of 27 778 Hz, 32K complex data sets, WALTZ16 decoupling during acquisition only, and 32K scans. Spectra were exponentially multiplied (line broadening, 5 Hz) prior to Fourier transformation.

Results and Discussion

The six poly(butyl methacrylate) samples were analyzed under two different GPC conditions and also by MALDI/TOF mass spectrometry. Low-angle laser light scattering (LALLS) was also applied to two samples of higher M_w. The results of these measurements are summarized in Table 1.

Table 1. Weight-Average Molecular Weight[a] of Poly(butyl methacrylate) Determined by GPC, MALDI, and LALLS

sample	GPC 1	GPC 2	MALDI	LALLS
1	6 210 (1.09)	4 170 (1.11)	3 880 (1.21)	ND[b]
2	9 420 (1.07)	6 150 (1.08)	5 870 (1.08)	ND
3	12 400 (1.07)	8 210 (1.11)	8 040 (1.06)	ND
4	37 200 (1.09)	27 000	32 200 (1.02)	32 500
5	85 400 (1.10)	69 200 (1.21)	77 800 (1.02)	72 600
6	109 000 (1.08)	79 000 (1.22)	94 400 (1.05)	ND

[a] The number in parentheses is the polydispersity index. [b] Not determined.

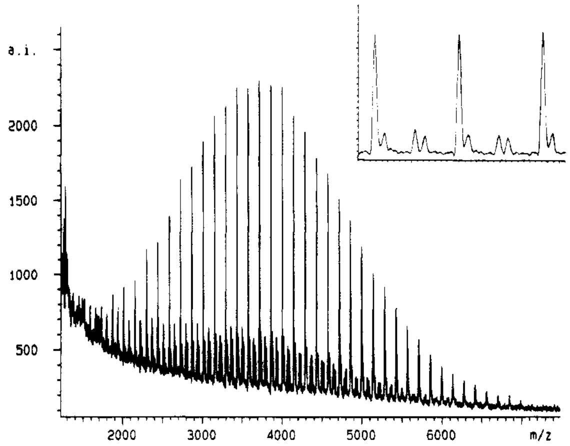

Figure 1. Positive ion MALDI/TOF mass spectrum of poly(butyl methacrylate) sample **1**. The inset shows an expanded view of the spectrum from mass 3400 to 3760. The data were acquired in the reflector mode and 5-point smoothed.

The positive ion MALDI/TOF mass spectrum of sample **1**, obtained with the reflector, is shown in Figure 1. A distribution is observed centered around *m/z* 4000, with a range extending from *m/z* 1500 to 7000. When we calculate for M_w, we obtain a value of 3880 with a polydispersity of 1.21. Clearly, MALDI is closer in agreement to the value provided by GPC 2. The peaks represent neutral molecular ions which have been cationized by sodium. This is the predominant form of ionization for non-acidic polymers[14,15] and is confirmed by the presence of a potassium cationized peak 16 Da higher. The inset shows an expansion of this spectrum which reveals peaks separated by 142 Da, consistent with the butyl methacrylate monomer mass and confirming a poly(butyl methacrylate) was synthesized. The first peak in the inset has a *m/z* value of 3437 which corresponds to the oligomer with 24 monomer units. This leaves a remainder of 29 Da for the end group which is accounted for as follows: 23 Da for the sodium, 2 Da for the end group (saturated end group is expected from the GTP polymerization used here), and 4 Da from contributions of ^{13}C (1.1% of abundance of ^{12}C) and the mass defect of H (^{1}H = 1.00783 Da). This calculation with several other peaks gives the same result for the end group. At this mass the instrumental accuracy is about 0.5 Da. Therefore, MALDI is able to confirm the monomer composition and saturated end group for this polymer.

Of interest are the smaller peaks which appear between the large molecular ions and correspond to a loss of 74 Da from the molecular ion. These small peaks may be due to (1) a neutral loss of butanol from the poly(butyl methacrylate) or (2) an end group different from that expected. For determination of a separate end group, ^{13}C, ^{13}C DEPT, and ^{1}H NMR were performed on this sample (data not shown). The ^{13}C and ^{1}H NMR

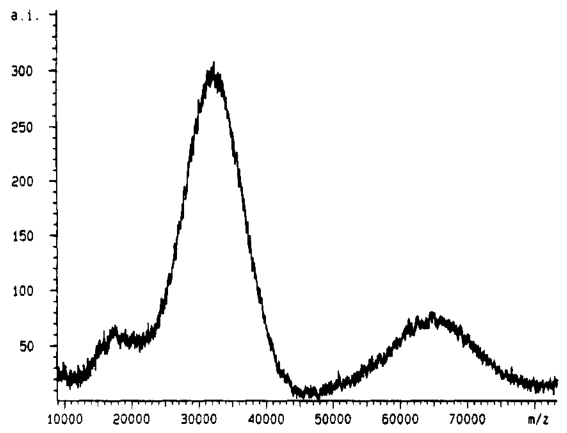

Figure 2. Positive ion MALDI/TOF mass spectrum of poly(butyl methacrylate) sample **4**. The data were acquired in the linear mode and 11-point smoothed.

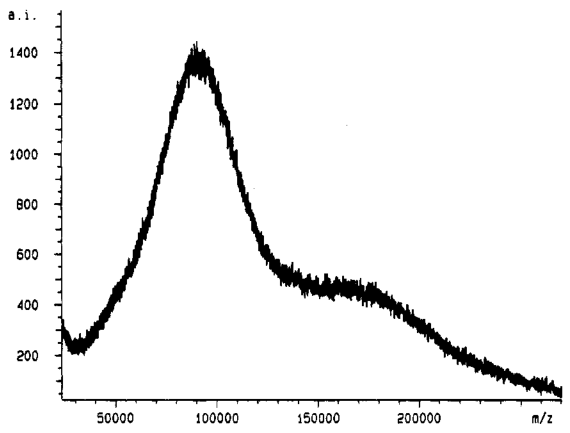

Figure 3. Positive ion MALDI/TOF mass spectrum of poly(butyl methacrylate) sample **6**. The data were acquired in the linear mode and 5-point smoothed.

spectra contain peaks for the saturated butyl methacrylate end group only. The ^{13}C DEPT experiment confirmed the methylene and methine carbons assigned as the end-group peaks in the ^{13}C spectrum. There are no extra peaks in the vinyl, carbonyl, or aliphatic region of any spectrum. Since the NMR does not show additional end groups, we assume a neutral loss of butanol occurs during the ionization and acceleration of the molecule. We observe the loss of butanol for all the poly(butyl methacrylate) samples where individual molecular ions were resolved. The neutral losses seem to be a single elimination of butanol because the distribution does not continue to low masses which would indicate severe fragmentation or multiple butanol loss. We postulate the terminal butyl ester is responsible for this loss. The loss of the proton on the terminal methine carbon can combine with the butoxy moiety to produce a neutral loss of butanol and leave a ketene on the polymer chain. Indeed, this reaction has been observed in solution chemistry with base catalysis.[25,26] Note that only one carbon in the polymer chain is a methine and is consistent with a single elimination of butanol. Small neutral losses such as this are not unusual; we have also observed water loss in MALDI of poly(acrylic acid).[13]

The spectra obtained for poly(butyl methacrylate) samples **2** and **3** (obtained in the reflector mode) are similar in that they all show molecular ions separated by 142 Da and all show the neutral loss of butanol. Some doubly charged ions are also observed at lower masses in these spectra. Confirmation of these peaks is provided by their Δ *m/z* which is 72, half of the monomer mass. The values we obtain for M_w (and polydispersity) for samples **2** and **3** are 5870 ($M_w/M_n = 1.08$) and 8040 ($M_w/M_n = 1.06$), respectively. For these polymers, we are also in closer agreement to values provided by GPC 2 (Table 1). One assumption made in these measurements is that the MALDI detector response is constant over the mass range of the polymer distribution. This is supported by recent work[27] which has shown that a 1:1 molar mixture of oligomers of methyl methacrylate with masses of 2500 and 5000 gave equal response in the MALDI/TOF mass spectrometer.

The spectrum obtained in the linear mode for sample **4** is displayed in Figure 2. Note there is not sufficient resolution at this mass to resolve the individual molecular ions; thus, the molecular ions convolute and become a continuous distribution. The spectrum shows three different charge states centered at about *m/z* 17 000, 32 000, and 65 000. The *m/z* 17 000 distribution represents the doubly charged ions, while the distribution at *m/z* 65 000 corresponds to dimers formed by aggregation of two polymer chains. The *m/z* 65 000 distribution cannot represent the singly charged polymer distribution because the *m/z* 17 000 would not correspond to triply charged ions. Likewise the converse is true, the *m/z* 17 000 cannot be singly charged ions as the *m/z* 65 000 distribution cannot be the trimer. As long as three charge states are present, the singly charged distribution can be determined, much like electrospray mass spectrometry. Also like electrospray, each distribution could be used as an independent confirmation of molecular weight.[28,29] From Table 1 the value of M_w determined by MALDI is in excellent agreement with the LALLS and in good agreement with both GPC values.

The spectrum obtained for sample **6** in the linear mode (Figure 3) shows two distributions centered around *m/z* 95 000 and 190 000. Since this spectrum only contains two distributions, it is difficult to tell which one corresponds to the singly charged ions. In these cases, GPC provides crucial information. Since the GPC measurements (Table 1) gave M_w of 109 000 and 79 000, we conclude that the distribution at approximately *m/z* 95 000 represents the singly charged ions and *m/z* 190 000 represents the dimer. From this singly charged distribution MALDI yields a M_w value of 94 400. This same argument was used for sample **5** (Table 1) where agreement between MALDI and GPC is reasonable. Additionally, LALLS on sample **5** gave a value of $M_w = 72\,600$, comparing well with the MALDI value of 77 800. Although the relative detector response at these higher masses has not been directly measured, the close agreement between the MALDI and LALLS M_w values suggests that the effect of detector bias is minimal.

It is important to note that in samples **4**–**6** the polydispersity index measured by MALDI is significantly lower than that from GPC. This is probably because the GPC data were not corrected for band broadening,[30–32] which, according to Hamielec,[33] can cause the polydispersity index to be 5–10% above the true value. Consideration of this effect would lead to reasonable agreement between values determined by MALDI and GPC. Furthermore, the MALDI results require no correction for this effect and may offer a more accurate measurement of polydispersity than GPC.

In Table 1 it is clear that M_w determined by MALDI is closer to values provided by GPC 2 for the first three samples, while values for the last three samples are intermediate between the GPC 1 and GPC 2 results. This demonstrates the utility of MALDI to provide accurate molecular weight and distribution information for those cases where some ambiguity may exist. This is corroborated by the light scattering results where MALDI is in excellent agreement. In addition, MALDI was able to provide specific structural information in the form of monomer and end-group composition on the first three samples.

Conclusions

The analysis of synthetic organic polymers by MALDI/TOF mass spectrometry provides values for the absolute molecular weight as well as shows the entire distribution of polymer species. In addition, the amount of organic solvents used for this technique (microliters) is greatly reduced compared to standard techniques such as GPC (liters). In this case, MALDI/TOF combined with GPC provided absolute molecular weights, composition, and end-group information for the poly(butyl methacrylate) synthesized by group-transfer polymerization. MALDI/TOF data were obtained from submilligram quantities of sample and in less than 15 min. The information provided by MALDI plays an important role in determining properties of a polymer and will be particularly important for the analysis of materials such as copolymers, grafted polymers, and other highly architectured polymers[34] for which no standard materials exist.

Acknowledgment. The authors are grateful to F. J. Huby for the light scattering analyses, to S. K. Wolk for the NMR analyses, and to both for their helpful discussions. We thank R. J. Butera for his GPC analysis and Professors Zhenwen Han and Ping Ping Wu (East China University of Science and Technology) for supplying the synthesized butyl methacrylate samples and their GPC analysis. D.E.K. and P.O.D. also thank Rohm and Haas for support of this research. D.T.W. and W.J.S. thank DuPont for permission to publish this work.

References and Notes

(1) Hillenkamp, F.; Karas, M.; Beavis, R. C.; Chait, B. T. *Anal. Chem.* **1991**, *63*, 1193A.
(2) Karas, M.; Bahr, U.; Giessmann, U. *Mass Spectrom. Rev.* **1992**, *10*, 335.
(3) Karas, M.; Hillenkamp, F. *Anal. Chem.* **1988**, *60*, 2299.
(4) Tanaka, K.; Waki, H.; Ido, Y.; Akita, S.; Yoshida, Y.; Yoshida, T. *Rapid Commun. Mass Spectrom.* **1988**, *2*, 151.
(5) Beavis, R. C.; Chait, B. T. *Rapid Commun. Mass Spectrom.* **1989**, *3*, 432.
(6) Beavis, R. C.; Chait, B. T. *Anal. Chem.* **1990**, *62*, 1836.
(7) Hillenkamp, F.; Karas, M.; Ingendoh, A.; Stahl, B. In *Biological Mass Spectrometry*; Burlingame, A., McCloskey, J. A., Eds.; Elsevier: Amsterdam, The Netherlands, 1990; p 40.
(8) Spengler, B.; Pan, Y.; Cotter, R. J.; Kan, L.-S. *Rapid Commun. Mass Spectrom.* **1990**, *4*, 99.
(9) Huth-Fehre, T.; Gosine, J. N.; Wu, K. J.; Becker, C. H. *Rapid Commun. Mass Spectrom.* **1992**, *6*, 209.
(10) Mock, K. K.; Davey, M.; Cottrell, J. S. *Biochem. Biophys. Res. Commun.* **1991**, *177*, 644. .
(11) Stahl, B.; Steup, M.; Karas, M.; Hillenkamp, F. *Anal. Chem.* **1991**, *63*, 1463.
(12) Castro, J. A.; Koster, C.; Wilkins, C. *Rapid Commun. Mass Spectrom.* **1992**, *6*, 239.
(13) Danis, P. O.; Karr, D. E.; Mayer, F.; Holle, A.; Watson, C. H. *Org. Mass Spectrom.* **1992**, *27*, 843.
(14) Bahr, U.; Deppe, A.; Karas, M.; Hillenkamp, F.; Giessmann, U. *Anal. Chem.* **1992**, *64*, 2866.
(15) Danis, P. O.; Karr, D. E. *Org. Mass Spectrom.* **1993**, *28*, 923.
(16) Danis, P. O.; Karr, D. E.; Westmoreland, D. G.; Piton, M. C.; Christie, D. I.; Clay, P. A.; Kable, S. H.; Gilbert, R. G. *Macromolecules* **1993**, *26*, 6684.
(17) Abate, R.; Ballistreri, A.; Montaudo, G.; Garozzo, D.; Impallomeni, G.; Critchley, G.; Tanaka, K. *Rapid Commun. Mass Spectrom.* **1993**, *7*, 1033.
(18) Burger, H. M.; Muller, H.-M.; Seebach, D.; Bornsen, K. O.; Schar, M.; Widmer, H. M. *Macromolecules* **1993**, *26*, 4783.
(19) Creel, H. S. *Trends Polym. Sci.* **1993**, *1*, 336.
(20) Danis, P. O.; Karr, D. E. *Macromolecules*, accepted for publication.
(21) Webster, O. W.; Hertler, W. R.; Sogah, D. Y.; Franham, W. B.; Rajan Babu, T. V. *J. Am. Chem. Soc.* **1983**, *105*, 5706.
(22) Dicker, I. B.; Cohen, G. M.; Farnham, W. B.; Hertler, W. R.; Laganis, E. D.; Sogah, D. Y. *Macromolecules* **1990**, *23*, 4034.
(23) Ainsworth, C.; Chen, F.; Kuo, Y. N. *J. Organomet. Chem.* **1972**, *46*, 59.
(24) Ernst, R. R.; Bodenhausen, G.; Wokaun, A. *Principles of Nuclear Magnetic Resonance in One* and *Two Dimensions*; Clarendon Press: Oxford, U.K., 1987; Chapter 4.
(25) Haener, R.; Laube, T.; Seebach, D. *J. Am. Chem. Soc.* **1985**, *107*, 5396.
(26) Seebach, D.; Amstutz, R.; Laube, T.; Schweitzer, W. B.; Dunitz, J. D. *J. Am. Chem. Soc.* **1985**, *107*, 5403.
(27) Larsen, B. S.; McEwen, C. N.; Simonsick, W.; Peacock, P. M. In *Proceedings of the 42nd ASMS Conference on Mass Spectrometry and Allied Topics*; Chicago, IL, May 29–June 3, 1994; ASMS: Santa Fe, 1994; p 518.
(28) Mann, M.; Meng, C. K.; Fenn, J. B. *Anal. Chem.* **1989**, *61*, 1702.
(29) Smith, R. D.; Loo, J. A.; Edmunds, C. G.; Barinaga, C. J.; Udseth, H. R. *Anal. Chem.* **1990**, *62*, 882.
(30) Tung, L. H.; Runyon, J. R. *J. Appl. Polym. Sci.* **1969**, *13*, 2397.
(31) Tung, L. H. *J. Appl. Polym. Sci.* **1969**, *13*, 775.
(32) Yau, W. W.; Kirkland, J. J.; Bly, D. D. *Modern Size-Exclusion Liquid Chromatography*; John Wiley and Sons: New York, 1979; Chapter 3.
(33) Hamielec, A. E. *J. Appl. Polym. Sci.* **1970**, *14*, 1519.
(34) Webster, O. W. *Science* **1991**, *251*, 887.

MA9411033