

Examination of the Covalent Cationization Method Using Narrow Polydisperse Polystyrene

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ABSTRACT: Four bromine- and phosphonium-terminated polystyrenes were analyzed by matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS) using covalent cationization. Bromine-terminated polystyrenes were prepared by atom transfer radical polymerization (ATRP) giving narrow molar mass (MM) distributions with number-average molar mass ranging from 2 to 40 ku. The resultant bromine end group was converted to a phosphonium ion. Precharging the polymer by covalent cationization is an alternative approach to traditional metal cationization for MALDI–MS polymer analysis. MALDI–MS, gel permeation chromatography, and proton nuclear magnetic resonance analyses confirmed ATRP synthesis and end group modification of the polystyrenes. High performance liquid chromatography at the critical point of adsorption was used to determine a phosphonium salt conversion between 37% and 90%. Similar MMs and MM distributions determined by MALDI–MS analysis were observed for Br-terminated polystyrene ionized using traditional metal cationization and the covalently cationized (i.e., phosphonium-terminated) polystyrene. Covalently cationized samples yielded better data quality than the Br-terminated samples. Higher signal intensity and signal reproducibility were observed for the covalently cationized samples using milder laser conditions.

Introduction

Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS) is a promising method for measuring accurate molar masses (MM) and molar mass distributions (MMD) of synthetic polymers.^{1–6} MALDI MS offers mass characterization of high molar mass polymers with minimal fragmentation and enables confirmation of polymer repeat unit and end group composition. In addition, valuable information on the nature of the polymerization process and the resultant architecture for many polymer types can be determined.

Molar mass determination by MS analysis requires the formation of intact macromolecular ions in the gas phase. Traditional synthetic polymer cationization involves association of a metal cation such as sodium, potassium, silver, and copper with certain functional groups present on the polymer such as polar, unsaturated, or aromatic groups. The typical MALDI MS protocol commixes a salt of one of the metals listed, an energy-absorbing matrix, and a polymer analyte. Reduced analyte signal often results from complications during the metal ionization processes in the gas-phase including competition between the analyte and matrix for cationization, self-clustering of the metal salts, and clustering of metal salts with matrix.^{7–10} One approach to increase the polymer ion signal is to apply more laser power; however, this may result in fragmentation of the polymer leading to a MMD that is not representative of the true distribution.⁵

An alternative approach is to covalently bond an organic species to the polymer forming an organic salt.^{11–16} These types of end group functionalization methods reduce the problems associated with metal ionization since no metal salt addition is required for polymer ionization. In addition, precharging the polymer is particularly useful for synthetic polymers lacking functionalities that complex with metal cations. Our approach has been to covalently bond a tertiary phosphine or amine to form a phosphonium or ammonium salt, thereby covalently attaching a single charge on the chain end of polyolefins, such as, polyethylene and hydrogenated polybutadiene derivatives.^{13,15} Similar chemistry was used by Coessens and Matyjaszewski to ionize low molecular mass polystyrene and poly(methyl methacrylate),^{11,17} while Ji and co-workers demonstrated that sulfonation chemistry can be used to form negatively charged polyisobutylene and polystyrene.^{15,16} More recently, Cox, Johnston, and Dasgupta generated quaternary amine end-functionalized polystyrene.¹⁴

In the previous study of MALDI–MS characterization of covalently cationized polyethylene, an upper detectable mass limit was observed (15000 g/mol or u).¹³ In addition, molar masses for polyethylene and some polyisobutylene samples determined by MALDI–MS were low compared to those determined via classical methods, such as gel permeation chromatography (GPC), osmometry, and nuclear magnetic resonance spectroscopy (NMR).^{13,15} Distortion in the distribution shape was also observed for the polyethylene samples. Negative deviation of MM moments might be attributed to MALDI-induced fragmentation or complications with the end group functionalization method.

In order for chain-end derivatization, in our case covalent cationization, to be a viable technique for polyolefin ionization in MS analysis, covalent cation-

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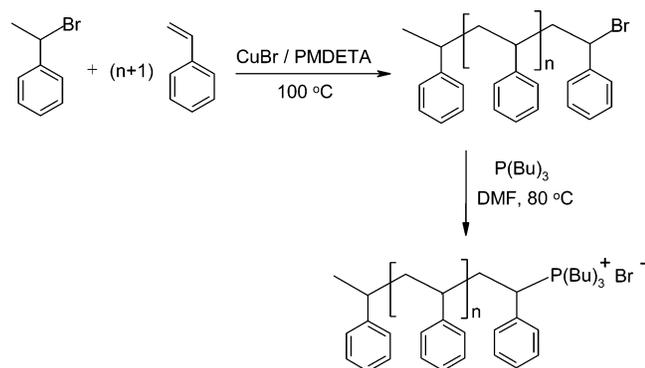


Figure 1. Synthesis of Br-terminated polystyrene by atom transfer radical polymerization, followed by substitution of bromine with tri-*n*-butylphosphine.

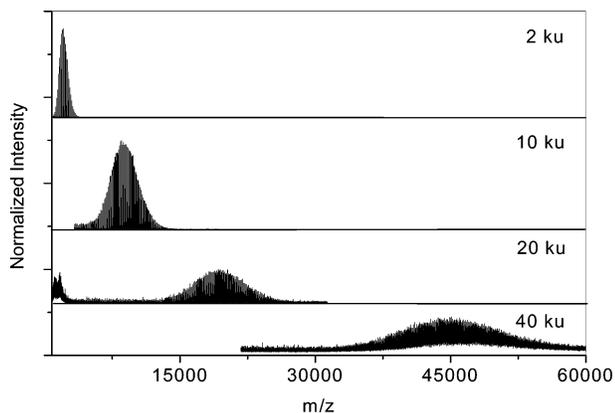


Figure 2. MALDI-MS of polystyrene distributions terminated with tri-*n*-butylphosphine where data are shifted vertically for clarity.

ization must provide accurate and reliable molar mass information over a mass range of interest. This can be achieved by applying the method to a well-characterized polymer. Polystyrene is an ideal choice since it is well characterized by both MALDI and traditional methods with numerous studies described in the literature showing characterization of polystyrene standards with narrow polydispersity index (PDI) < 1.2.^{4,6,18}

In the present study, four narrow polydispersed (PDI < 1.2) polystyrenes with number-average molar mass ranging from 2 to 40 ku were synthesized and subsequently modified using the covalent cationization method (Figures 1 and 2). We demonstrate that the method can be used for ionization of a broad mass range. GPC and ¹H NMR were performed to verify polymerization. ¹H NMR and MALDI-MS confirmed reaction conversion of the derivatized samples, and high performance liquid chromatography (HPLC) at the critical point of adsorption (CPA) was used to determine percent covalent cationization.

Experiment

Materials. HPLC grade tetrahydrofuran (THF) and acetonitrile (ACN) were purchased from J. T. Baker (Phillipsburg, NJ).³⁸ Chloroform-*d* was purchased from Acros (Morris Plains, NJ). All other reagents were purchased from Aldrich (St. Louis, MO). Reagents were used as is unless otherwise indicated. Copper bromide (Cu^IBr) was purified by stirring in acetic acid overnight, filtering to collect the solids, and washing with ethanol. Styrene monomer (Sty) was passed over neutral alumina to remove inhibitor.

Polystyrene Synthesis via Atom Transfer Radical Polymerization (ATRP). Reagent amount, reaction condi-

Table 1. Reaction Conditions and Conversion for Atom Transfer Radical Polymerization Synthesis of Br-Terminated Polystyrene

series	sty:BEB:CuBr:PMDETA ^a (mole ratio)	styrene (mL)	reaction time (h)	conversion (%)
2	20:1:0.2	10	5	72
10	100:1:0.5	10	6	88
20	200:1:0.5	10	9	83
40	600:1:0.5	15	48	91

^a Sty represents styrene, BEB represents 1-bromoethylbenzene, and PMDETA represents *N,N,N',N',N''*-pentamethyldiethylenetriamine.

tions, and conversions for ATRP synthesis are given in Table 1. Reagent quantities, reaction conditions, and characterization results for synthesis of Br-terminated polystyrene $M_n \approx 2$ ku are given below and in the Results. Synthesized polystyrene samples will be referred to in the text as 2 ku, 10 ku, 20 ku, and 40 ku corresponding to the target molecular mass moment. Cu^IBr (0.121 g, 8.75×10^{-4} mol) and Sty (10 mL, 0.0875 mol) were added to a 25 mL round-bottom flask with stir bar, which was then sealed with a rubber septum and secured with copper wire. The contents of the flask were stirred for 45 min at room temperature with argon purged through the solution. *N,N,N',N',N''*-Pentamethyldiethylenetriamine (PMDETA, 0.180 mL, 8.75×10^{-4} mol), used for the formation of the catalyst complex with Cu^IBr, was introduced to the flask using a microliter syringe. The mixture was stirred an additional 15 min until the solution became homogeneous and had a yellow-green color indicating formation of the catalyst. After removing the argon and vent lines, the flask was transferred into a 100 °C oil bath. The initiator 1-bromoethylbenzene (BEB) was added to the flask, and then the polymerization was allowed to continue for duration dependent on the desired molecular mass (Table 1). Removing the flask from the heated oil bath and exposing the contents to air stopped the reaction. The product was dissolved in toluene, stirred over DOWEX ion-exchange resin overnight, and filtered through neutral alumina to remove the copper catalyst. The collected effluent was precipitated twice in methanol and once in hexanes to remove residual styrene and catalyst, and finally dried in a vacuum oven overnight at 45 °C. Polymerization and Br-termination were confirmed by GPC and ¹H NMR analyses (88% conversion for 2 ku sample).

End Group Modification of Bromine with Tri-*n*-butylphosphine. Bromine-terminated polystyrene (500 mg, 5.7×10^{-5} mol) in 10-mol excess of tri-*n*-butylphosphine (TBP, 0.13 mL, 5×10^{-4} mol) was dissolved in dimethylformamide (DMF, 5 mL, 0.06 mol) at 80 ± 2 °C. Reagent quantities, reaction conditions, and characterization results for TBP-derivatization of Br-terminated polystyrene $M_n \approx 10$ ku are given here and in the Results. The reaction was allowed to proceed for 2 d after which the hot polymer solution was precipitated in cold methanol (≈ 200 mL) and then filtered. To remove excess TBP and TBP oxide, the filtered product was redissolved in toluene, gently reheated to 60 °C, reprecipitated in cold methanol, and filtered twice more (40% recovery for 10 ku). Bromine conversion to TBP was confirmed by ¹H NMR and MALDI-MS. Product yields were determined by HPLC at CPA (90% product yield for 10 ku).

Polymer Characterization. ¹H NMR. All measurements were performed at 25 °C. Each sample contained between 5% and 10% by mass polystyrene in chloroform-*d*. Resonance frequency of the internal ¹H chemical shift reference, tetramethylsilane (TMS), was set to 0 ppm for both ¹H NMR systems. Polymer conversion analysis for the ATRP synthesis was determined by ¹H NMR (GSX 270, JEOL USA, Inc., Peabody, MA). Experiments used were standard JEOL implementations of 1D-proton acquisition. Recycling delay of 20 s and a 45° excitation pulse were used to obtain accurate intensity measurements. 1D spectra were recorded at 270.17 MHz using 128 or 256 scans of 16384 data points and a spectral width of 15 ppm.

End group characterization and M_n values for the Br-terminated polystyrene were determined by ¹H NMR (DRX-

600, Bruker, Billerica, MA). The spectrometer was equipped with a 5 mm triple resonance cryoprobe with ultrahigh sensitivity. Experiments used were standard Bruker implementations of 1D-proton acquisition. Recycling delay of 10 s and a 90° excitation pulse were used to obtain accurate intensity measurements. The 1D spectra were recorded at 600.13 MHz using 256 scans of 16384 data points and a spectral width of 10 ppm.

GPC. Polymer MMD and its moments were measured using a GPC system from Waters Technologies (Milford, MA) equipped with a 1515 isocratic HPLC pump with online degassing, 717 Plus autosampler, 2414 refractive index detector, a guard column, and two mixed bed columns (HR4 and HR4E) with 5 μm particle size. The HR4 is specified for molar mass separation range of 5000–600000 g/mol and the HRE4 a 50–100000 g/mol range for polystyrene. The separation was performed in THF at 1 mL/min nominal flow rate at 30 °C with a sample concentration of 2.5 mg/mL. The injection volume was 20 μL with a volume fraction of 0.1% anisole as an internal reference peak. The column set was calibrated by a series of narrow polydispersity polystyrene standards spanning the mass range of the prepared samples.

HPLC at CPA. HPLC separations of polystyrene were performed on an Alliance 2695 Separations Module equipped with on-line Fraction Collector III and column heater (Waters) maintained at 35 °C. The module provides a low-pressure quaternary gradient pumping system with a lag volume of 0.6 mL to the column inlet, online solvent degassing, and automated sample injection from 2 mL vials. Two on-line detectors were used: evaporative light-scattering detector (ELSD) PL-ELS 1000 (Polymer Laboratories, Shropshire, U.K.) and Waters 2487 dual wavelength absorbance spectrophotometer. A Nova-Pak C₁₈ reversed-phase column (300 mm \times 3.9 mm) with 4 μm particle size from Waters was used for separation of polystyrene samples in THF/ACN mixtures in both gradient and isocratic modes. Gradient separations were performed at a flow rate of 1 mL/min using linear 10 min gradient of 100% ACN to 100% THF. Flow rates for isocratic separations were 0.25 mL/min. All samples for HPLC experiments were prepared at 1 mg/mL concentration in THF for gradient experiments and in the mobile phase for isocratic experiments. Waters Empower software was used for data acquisition, data reduction, and peak deconvolution.

MALDI-MS. The MALDI matrix, all-trans retinoic acid, and polystyrene were mixed dry (50 mg and 1 mg, respectively). The mixture was dissolved in 1 mL THF. For the measurements using the traditional metal cationization method, silver trifluoroacetate (AgTFA) was used as the cationizing reagent in a 1:1 by volume ratio of AgTFA solution (0.5 mg/mL THF) and PS/retinoic solution. All MALDI samples were deposited on the target by electrospray. The MALDI-TOF MS was performed on a Bruker (Billerica, MA) REFLEX II at a typical operating pressure of $\sim 10^{-5}$ Pa.¹⁹ Samples were irradiated using 337 nm N₂ laser. Mass spectra were acquired in the reflectron mode at 25 kV using delayed extraction and low-mass (i.e., matrix-ion) blanking. Each spectrum shown is the sum of at least 256 discrete laser shots and is shown without smoothing or background subtraction except where specified. The estimated standard uncertainty in overall signal intensity from repeatability studies is 15%.

The MALDI-MS instrument was mass calibrated using Sequazyme peptide standards (Applied Biosystems, Foster City, CA) spanning the m/z region of interest. MALDI-MS data were processed using the Polymerix software package from Sierra Analytics (Modesto, CA). Data used to tabulate results were integrated and centroided. From these data, the homopolymer series and end groups were annotated by comparing the labeled polymer peaks to the theoretical masses of possible oligomers in the polymer series. The program used the C-12, Ag-107, and Br-79 isotopes for mass calculations. Polymerix was also used to determine the M_n , M_w , and PDI of the polymer distributions as related to the end group where assessable.

Table 2. Molecular Mass Values (g/mol) of Br-Terminated (Br-PS) and Tributylphosphine-Terminated Polystyrene (TBP-PS) Series Determined by GPC

series	Br-PS		TBP-PS	
	$M_n^{a,b}$	PDI	$M_n^{a,b}$	PDI
2	1950 \pm 60	1.06	2050 \pm 80	1.04
10	6940 \pm 340	1.06	6640 \pm 430	1.10
20	15150 \pm 285	1.05	15120 \pm 420	1.06
40	35945 \pm 1390	1.07	34360 \pm 1100	1.14

^a M_n values shown are subject to larger uncertainty than M_w due to the fact that the detection method is mass dependent.

^b Estimated expanded uncertainty arises from choice of baseline, reference peak shift monitored over multiple injections, and repeatability studies over two experiment days.

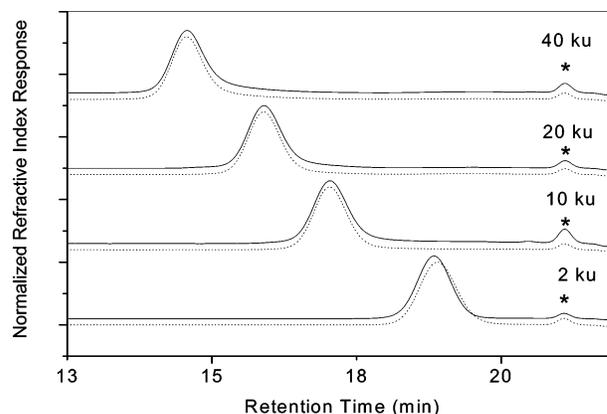


Figure 3. GPC traces of Br-terminated (solid line) and tributylphosphine-terminated (dashed line) polystyrene samples where data are shifted vertically for clarity, 0.1% anisole (*) was used as an internal reference peak.

Results

In the present study, four narrow polydisperse polystyrene distributions were synthesized with M_n ranging from ≈ 2 to 40 ku and will be referred to as 2 ku, 10 ku, 20 ku, and 40 ku. Figure 1 shows the reaction scheme for the polystyrene samples synthesized. Using ATRP to prepare the polystyrene systems was a natural choice since the synthesis yields polymers of narrow polydispersity necessary for optimum MALDI characterization. Other advantages of ATRP are its tolerance to impurities and its molecular mass range control.^{20–22} The BEB initiator generates a bromine-terminated polystyrene (Br-PS). The α end is chemically inert, while the halogenated ω end can be easily replaced to form other functionalities. Substitution of the bromine with trialkylphosphine produces a single positive charge on each polymer chain that allows direct detection by MALDI-MS without the addition of metal salt for ionization.^{11,17,23} Figure 2 shows MALDI spectra of the TBP-terminated polystyrene (TBP-PS) distributions.

GPC, solution ¹H NMR, HPLC at CPA, and MALDI-MS were used to confirm synthesis of Br-PS and subsequent derivatization to form TBP-PS products.

GPC. GPC analysis verified ATRP synthesis of polystyrene. Examination of the molar mass moments and polydispersities determined from the GPC analysis (Table 2) confirms that well-defined polymer distributions with polydispersities less than 1.2 were generated. Furthermore, subsequent TBP modification of the Br end group did not change the overall distribution shape of the polystyrene samples as shown in Figure 3.

¹H NMR. NMR analysis of the sample removed directly from the reaction flask shows high polymer

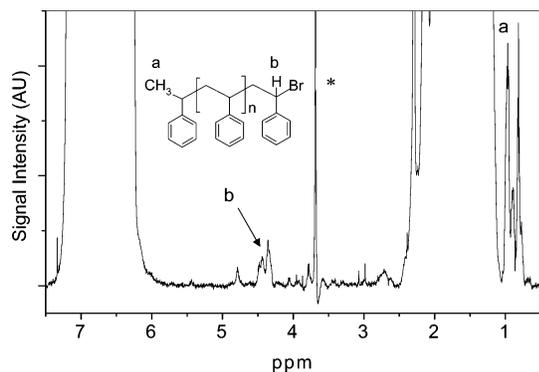


Figure 4. 600 MHz ^1H NMR spectrum of Br-terminated polystyrene 20 ku (see Results ^1H NMR section for chemical shift assignments), where * represents the center of spectrum peak.

Table 3. Molecular Mass Values (g/mol) of Br-Terminated Polystyrene (Br-PS) Series Determined by ^1H -NMR^a

series	M_n , methine H at ω end ^b	M_n , CH_3 Hs at α end ^c
2	2250 ± 150	2060 ± 150
10	10130 ± 1000	10170 ± 1000
20	23740 ± 3300	19900 ± 3300
40	51620 ± 5000	45100 ± 5000

^a The estimated expanded uncertainty reported here arises from the choice of baseline in the peak integration complicated by proximity of dominant neighboring peaks and the presence of small amounts of impurities. ^b M_n values were determined from the ratio of integrals for the phenyl ring protons to the methine proton geminal to the bromine at the ω -end. ^c M_n values were determined from the ratio of integrals for the phenyl ring protons to the methyl protons at the α -end.

conversion for the ATRP synthesis of the four polystyrene distributions (Table 1). The percent polymer conversion was calculated by dividing the integral of the proton resonance corresponding to the aliphatic protons of polystyrene (ca. 1.1–2.5 ppm) by the sum of that integral and the integrals of the two resonances corresponding to two of the three vinyl protons of the styrene monomer (5.25 and 5.75 ppm).

NMR analysis also confirmed Br-termination of the polystyrene oligomers. Figure 4 shows the 600 MHz ^1H NMR spectrum of Br-PS 20 ku sample. Reasonable end group determination of the high molecular mass samples required the enhanced resolution and sensitivity of a 600 MHz instrument equipped with cryoprobe. The peak at 4.3–4.6 ppm is assigned to the methine proton geminal to the terminal bromine group, and the peak at 0.9–1.12 ppm is assigned to the terminal methyl group protons, associated with the initiator. Resonances corresponding to the methine proton and the terminal methyl group were protons observed for all four Br-PS samples. The expected ratio of the integrals of these two peaks is 1:3. Ratios for the 2 ku and 10 ku Br-PS were within 10% of the expected value, and within 20% for Br-PS 20 ku and Br-PS 40 ku. At high conversion percentages, undesired HBr elimination sometimes occurs;^{24,25} however, no resonances corresponding to termination by HBr elimination were observed in the NMR spectra for any of the Br-PS distributions.

The NMR-determined M_n values for the Br-PS series are given in Table 3. The estimated expanded uncertainty reported here arises from the choice of baseline in the peak integration complicated by proximity of dominant neighboring peaks and the presence of small amounts of impurities.

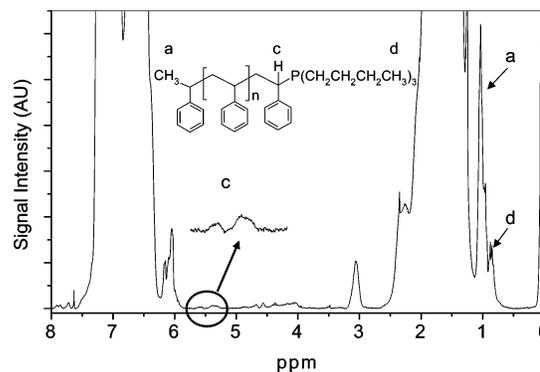


Figure 5. 270 MHz ^1H NMR spectrum of tributylphosphine-terminated polystyrene 2 ku (see Results ^1H NMR section for chemical shift assignments).

TBP substitution of the bromine end group is evident in part by loss of the peak corresponding to the methine proton geminal to the bromine (Figure 5). A broad peak at 0.7–0.9 ppm likely corresponds to the three terminal methyl groups of the TBP group. NMR spectra of TBP-PS 10, 20, and 40 ku showed the presence of residual unreacted TBP. The triplet corresponding to unreacted TBP (0.85 ppm) overlaps with the product resonance. A broad proton doublet at 5.6–5.8 ppm likely corresponds to the methine proton geminal to the TBP group. ^1H NMR analysis of the TBP-PS 2 ku sample (Figure 5) clearly shows resonances at 2.8–3.2 and 5.9–6.2 ppm, indicating the presence of a vinylic group. HBr elimination will lead to an undesired vinylbenzyl-terminated (vinyl-terminated) product.

Accurate determination of bromine conversion to TBP was complicated. We found that TBP-PS 2 ku sample was partially soluble in cold methanol. Only the high mass portion of the distribution was recovered by precipitation. End group analysis of the H NMR data provides accurate and reliable product yields. In the present study, the purification process and not the characterization tool is the limiting factor. Since the full TBP-terminated distribution was not recovered, the H NMR analysis of the precipitated TBP-PS sample shown in Figure 5 shows bias due to the precipitation.

For the higher mass samples, resonance corresponding to unreacted TBP interfered with the terminal methyl protons of the polymer-bound TBP. Furthermore, the proton geminal to the TBP group yielded a weak resonance too broad for quantitation. Therefore, product yields were determined using HPLC at CPA.

HPLC at CPA. Liquid chromatography at the critical point of adsorption has been successfully used to characterize compositional heterogeneity of complex polymers.²⁶ There are two competing factors at play in liquid separation of polymer systems. Size exclusion (steric effect) favors retention of low molecular mass species, while adsorption interactions of the repeat unit and the stationary phase favor retention of higher molecular mass species. At the CPA, these two factors completely compensate for each other in terms of retention. As a result, molar-mass independent separation can be achieved; therefore, macromolecules are separated based on chemical or microstructural differences and not mass. Near CPA conditions for a given polymer/stationary phase combination are usually observed using isocratic elution in a specific narrow range of mobile phase compositions, which can be further fine-tuned by adjusting the column temperature.²⁶ A new

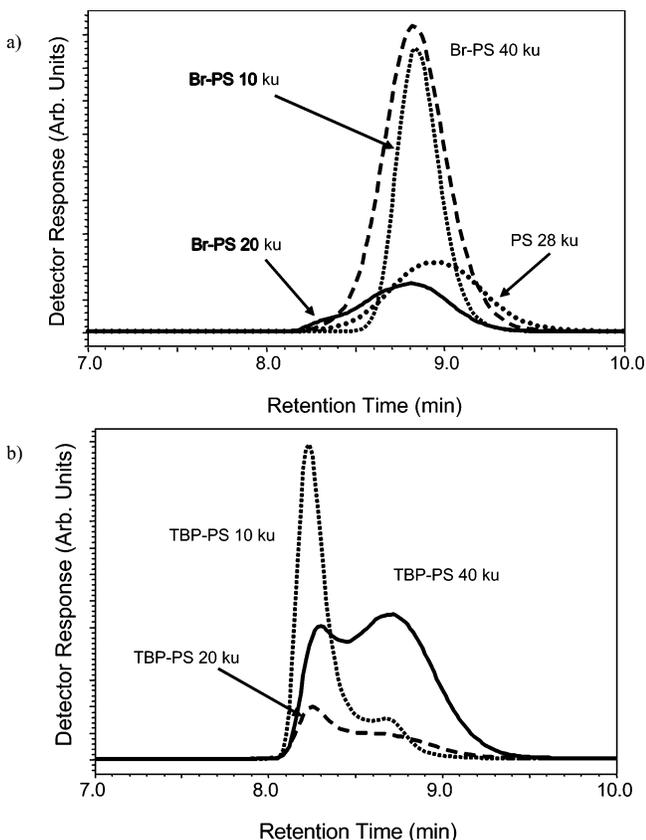


Figure 6. (a) Elution chromatograms at the critical point of absorption (CPA) obtained with an evaporative light scattering detector (ELSD) for Br-terminated PS (Br-PS) 10, 20, and 40 ku and a polystyrene standard with narrow polydispersity ($M_n = 28000$ u) (b) Elution chromatograms at CPA obtained with ELSD for tributylphosphine-terminated polystyrene (TBP-PS) 10, 20, and 40 ku.

approach of finding CPA through gradient elution was proposed recently.²⁷ Gradient separations at CPA have proven effective in determination of chemical composition distribution of copolymers and polymer blends.²⁷ However, isocratic separations at CPA were found to be the best choice when functional group distributions of telechelic polymers were under investigation.²⁶ Therefore, we used the isocratic method to separate and quantitate the products of the TBP-derivatized PS samples.

The polystyrene CPA in ACN/THF binary mobile phase using a reversed-phase C_{18} column corresponds to 48% THF and was determined previously by gradient and isocratic elution of narrow polydispersity polystyrene samples.²⁷ The latter method was used for analysis of Br-PS 10–40 ku, the respective TBP-derivatized samples, and an α -*sec*-butyl, ω -hydrogen terminated polystyrene standard ($M_n = 28000$ u, PDI < 1.1, purchased from Waters). Note that TBS-PS 2 ku sample was not included in the HPLC analyses because its low molar mass does not allow for complete elimination of molar mass dependence of retention even at the CPA.

Parts a and b of Figure 6 show the polystyrene chromatograms obtained using evaporative light scattering detection (ELSD). As can be seen (Figure 6a), polystyrenes of different molar masses with alkyl/hydrogen and hydrogen/bromine termination elute as unimodal peaks at similar retention times (8.9 and 8.8 min, respectively). The peak overlap of four samples

signifies the mass-independent separation. Figure 6b shows two elution peaks are observed for the TBP-PS samples. The later-retention peaks (8.7–8.8 min) coincide with the apex of Br-terminated polystyrene samples and the standard suggesting correspondence to the neutral vinylbenzyl-terminated polystyrene. The earlier peak has a lower retention (8.2 min) that is associated likely with the more polar TBP-bound polymer. These two separated fractions of the effluent were collected, and the end groups were confirmed by MALDI-MS. As the fractions with different end groups in TBP-PS samples were not baseline-separated, the corresponding peaks were deconvoluted using the assumption that each constituent at a given apex had a Gaussian shape with the same width. A relative ratio of the areas of the deconvoluted peaks was used to determine a TBP conversion of $90 \pm 5\%$ for TBP-PS 10 ku, $37 \pm 5\%$ for TBP-PS 20 ku, and $67 \pm 5\%$ for TBP-PS 40 ku (uncertainty given for deconvolution analysis). The difference in product yield may be attributed to variations in the reaction conditions (e.g., temperature conditions or slight differences in reagent concentrations).

Recently, Mengerink, Cramers, et al. reported that ELSD response could show nonlinearity with respect to concentration and molar mass for comparison of polymers with significantly different molar masses and peak areas.²⁸ However, in the present study, the molar masses and peak widths of TBP-PS samples should be similar since they are products from the same precursor polymer.

MALDI-MS. Mass spectrometry provides both polymer molar mass moments and compositional information. Figures 7a,b and 8a,b show the mass spectra of the Br-PS and TBP-PS 2 ku, respectively. The M_n values and polydispersities determined from the MALDI-MS data are listed in Table 4. Silver trifluoroacetate was used as a cationizing reagent for the Br-terminated polystyrene (Figure 7a), which generated a myriad of peak clusters. A closer examination (Figure 7b) shows that the Br-PS spectrum consists of three main peak series with a repeat unit corresponding to styrene (monoisotopic 104.0626 u). The origin of all three series was confirmed by analysis in the linear mode. End group analysis shows that series a corresponds to silver cationized Br-terminated polystyrene 186.72 ± 1.05 u (monoisotopic 186.8312 u). Series b, the most intense ion signal, corresponds to silver cationized vinyl-terminated polystyrene 211.82 ± 0.80 u (monoisotopic 210.9677 u), which likely results from an HBr loss from Br-PS. Series c is a composite peak mainly corresponding to sodiated vinyl-terminated polystyrene 127.42 ± 1.36 (monoisotopic 127.0524 u), which has been observed in the absence of a strong ionizing agent.²³ Since no elimination product was observed in the ^1H NMR analysis of Br-PS, the elimination products observed for peak series b and c are likely caused by the MALDI-MS process.

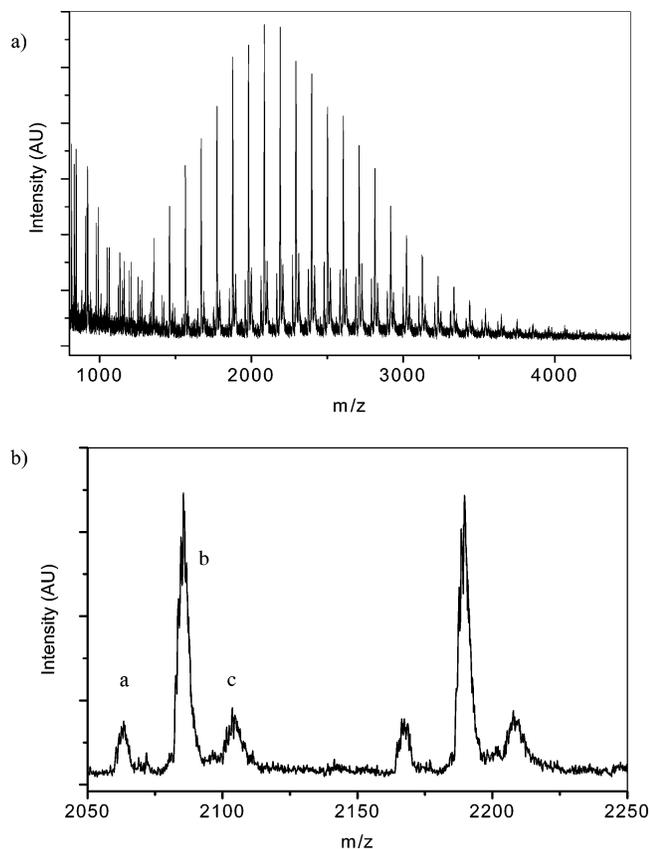
The values above include all data collected. Here, 186.79 ± 0.44 u, 211.13 ± 0.38 u, and 127.03 ± 0.46 u correspond to series a–c when two sets of data are removed due to one being associated with large degree of uncertainty and the second a statistical outlier. Removal of the two data sets brings the sample mean and standard deviation much closer to the theoretical value.

MALDI-MS analysis of the Br-PS 10 ku distribution (not shown) mirrors the results observed for the low

Table 4. Molecular Mass Values (g/mol or u) of Bromine-Terminated (Br-PS) and Tributylphosphine-Terminated Polystyrene (TPB-PS) Determined by MALDI-TOF MS

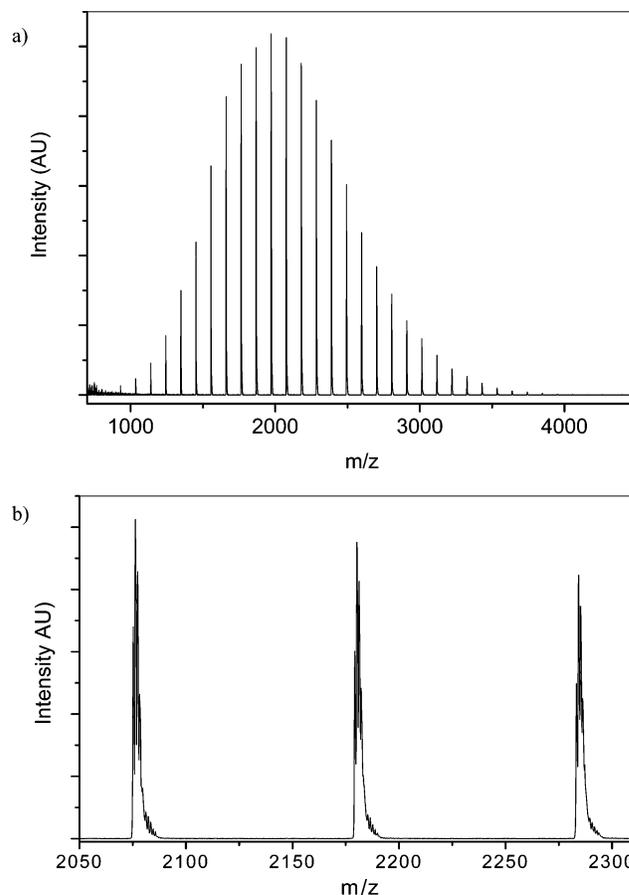
series	Br-PS		TBP-PS		TBP-PS with AgTFA Addition	
	$M_n^{a,b}$	PDI	M_n^b	PDI	$M_n^{b,c}$	PDI
2	2470 ± 50	1.06	2220 ± 70	1.04	2140 ± 30	1.04
	2185 ± 140	1.06				
10	9060 ± 115	1.04	8850 ± 180	1.04	8800 ± 120	1.04
	8100 ± 180	1.07				
20	15580 ± 450	1.14	18 550 ± 120	1.05	18 270 ± 100	1.06
40	42310 ± 2040	1.07	42 000 ± 2000	1.08	42 300 ± 1700	1.08

^a Top M_n value is determined from the intact Br-terminated series. Bottom M_n value is determined from the predominant series corresponding to HBr elimination from Br-PS. ^b The estimated expanded uncertainty for the molecular mass moments represents in part the statistical uncertainty (type A) and arises from repeatability studies, choice of baseline, and laser power. It however does not include instrument uncertainty (type B). ^c At least 10-mol excess AgTFA relative to polystyrene was used.

**Figure 7.** MALDI-MS of Br-terminated polystyrene 2 ku cationized with silver trifluoroacetate.

mass sample in that all three series were observed. Only one series was observed for Br-PS 20 ku and 40 ku. The series observed for the high molecular mass polystyrene samples likely a composite peak corresponding predominantly to series b since that peak predominates for the low mass samples; however, the mass resolution above m/z 15000 is not sufficiently mass-resolved to distinguish the intact Br-terminated and vinyl-terminated series.

Parts a and b of Figure 8 show the mass spectrum for the TBP-derivatized 2 ku polystyrene. The TBP-PS themselves are polymer-bound phosphonium salts; therefore, AgTFA addition was not necessary. Unlike the Br-PS spectrum, only one peak series is observed for the TBP-PS (Figure 8b) with peaks separated by styrene and end group mass 203.41 ± 0.36 u (monoisotopic 203.1929 u). No peaks corresponding to fragmentation were observed. TBP-PS 10, 20, and 40 ku samples also show one peak series in the respective mass spectrum. Unlike the low-mass analogues, the

**Figure 8.** MALDI-MS of tributylphosphine-terminated polystyrene 2 ku.

isotopic peak distribution and instrument effects at 20 and 40 ku masses introduce broadening that reduce the certainty of the end group assignments. Since PS cationized by sodium and/or potassium ions generates a weak observable in the absence of a strong cationizing agent, Br-PS in the absence of AgTFA was used as a baseline or control. For example, Br-PS 40 ku in the absence of silver produces a very weak while TBP-PS produces a strong signal lending support that the polymer ion is preformed.²³ Similar justification can be used for the 20 ku analogue.

As stated earlier, the TBP substitution reaction leads to partial conversion of Br-terminated polystyrene samples. The second end group results from HBr elimination giving a vinylbenzyl-terminated product. In the absence of a strongly cationizing agent (i.e., silver salt), the vinylbenzyl-terminated product will be negligible if detected at all by MALDI-MS. Addition of the

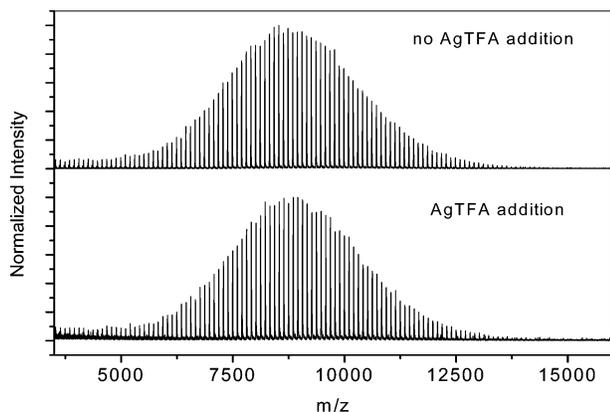


Figure 9. MALDI-MS of tributylphosphine-terminated polystyrene 10 ku sample with silver trifluoroacetate (AgTFA) addition shows no silver-cationized vinylbenzyl-terminated polystyrene series. Data are shifted vertically for clarity.

same agent to the resulting precharged TBP-PS and neutral vinylbenzyl-terminated polystyrene mixture provides an opportunity to evaluate the ionization efficiency of the neutral polystyrene product toward silver cationization relative to the precharged polymer. Figure 9 shows normalized mass spectra for the TBP-PS 10 ku sample with and without AgTFA addition. In this experiment, 50-mole excess AgTFA was added, but no silver cationized vinylbenzyl-terminated styrene series is observed.

Discussion

Three differences are immediately evident between the MALDI-MS data for the Br-PS and TBP-PS samples (Figures 7 and 8). First, the covalently charged styrene oligomers yield higher signal intensity under milder laser power conditions than the silver cationized Br-PS. Second, unlike the Br-PS spectrum, only one peak series is observed for the TBP-PS (Figure 7). Third, the TBP-PS spectrum is cleaner than the Br-PS spectrum.

The enhanced signal intensity observed for the TBP samples compared to Br-PS likely results from the charging species already being "attached" to the polymer. Therefore, no competition occurs for charging agent between the polystyrene and neutral matrix molecules during the MALDI process. As a result, only a small amount of energy is required for dissociation of the TBP-charged oligomer and Br⁻ counterion.

In the present study, the cationization efficiency of the covalently charged polystyrene is unity. We follow a similar assumption made by Cox, Johnston, and Dasgupta for the ionization efficiency of quaternary amine precharged polystyrene since the charge-moiety is directly "attached" to the polymer.¹⁴ Assuming unity for the cationization of the TBP-PS samples and similar desorption energies for the polystyrene, the Ag⁺ cationization efficiency of neutral Br-terminated and vinyl-terminated polystyrene appears to be less than one. In the 10 ku sample mixture in which both TBP-PS and vinylbenzyl-terminated polystyrene (9:1) is present, only the TBP-terminated oligomers were observed even with 50-mole excess of AgTFA added (Figure 9). The predominance of the TBP-PS was observed for all the TBP-PS samples doped with AgTFA over a range of laser energies (0.5–2 μ J, 3 ns pulse). Although the peak resolution is not sufficient to distinguish TBP charged and Ag⁺-charged vinyl-terminated oligomers for the 20

ku and 40 ku samples, the oligomer peak mass resolution for the 20 ku sample is similar for both with and without silver addition. The lack of peak broadening from the silver-cationized vinyl-terminated species suggests that the covalently cationized signal dominates the spectra. The previous ionization efficiency study by Cox et al. demonstrates that precharging the polystyrene yields an ionization efficiency an order of magnitude higher compared to the same polymer terminated with H, OH, and tertiary amine and then analyzed with silver cationization.¹⁴

Covalently cationized polystyrene samples yield strong MS signal well below the laser energy threshold for silver cationization of the Br-PS samples. Even at laser energy threshold (signal-to-noise ratio \approx 5:1), Br-PS samples were found to undergo laser-induced fragmentation. Immediate H-Br elimination at the ω -terminus leads to a stable vinylbenzyl group. However, the Br-PS 10 ku data show an increase in the low mass tail with increasing laser energy suggesting initial bromine loss followed by depolymerization. Subsequent hydrogen atom loss stops the fragmentation process. Similar results were observed previously for MALDI-MS analysis of halide-terminated polymers.^{29–31}

MALDI-MS of covalently cationized samples also produces cleaner spectra than the silver cationized Br-PS. Using milder laser conditions for TBP-PS samples reduces interference from matrix ion clusters. Gentler desorption conditions also minimize the presence of sodiated-oligomers. Since polystyrene has lower sodium cationization efficiency than for silver, no sodiated vinyl-terminated polystyrenes were observed for the silver doped TBP-terminated samples. The Br-PS and TBP-PS spectra collected with silver salt added clearly show a noisier baseline than the TBP-PS data collected without silver salt addition. The exclusion of metal salt, in this case AgTFA, also eliminates the chemical interference from metal and matrix adducts. Chemical noise peaks due to adduct formation and metastable ion decay sometimes plague polymer spectra collected in the reflectron mode.³² These interference peaks cause problems in the area of quantitative analysis and must be minimized.

The primary use of the covalent cationization method is to ionize polymers, such as polyolefins, that cannot be charged by traditional methods. However, covalent cationization may prove useful in the systematic studies for improving the MALDI method for polymers with broad polydispersities (PDI > 1.4) and asymmetric distributions, as well as the technique in general. Previously, equimolar blends of homopolymer standards with narrow polydispersity were used to characterize factors causing mass discrimination effects observed in MALDI-MS analysis of polydisperse polymers (PDI > 1.4).^{33–35} Each narrow polymer distribution can be well characterized and then combined to simulate a polymer with wide polydispersity. The covalent cationization method provides a unique opportunity to decouple the ionization and desorption in the MALDI process. Comparing MALDI-MS of a precharged polystyrene blend and the analogous polystyrene terminated with an inert end group and ionized by a metal salt provides information about the ionization and desorption processes occurring as a function of mass. The influence of the chemical nature of the end group on the desorption process may also be laid to rest by changing the functionality of the inert end group. A comparison study may also lend

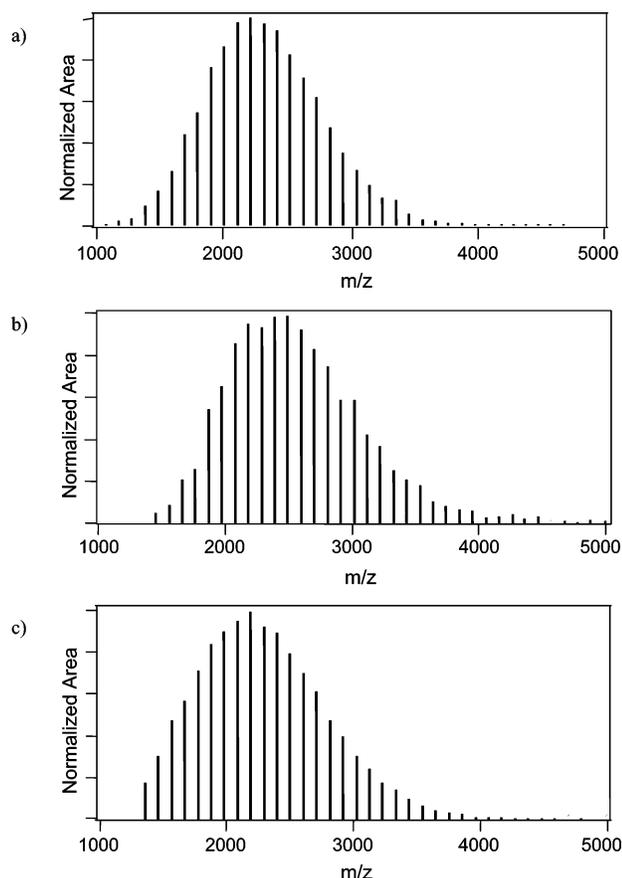


Figure 10. Centroid representation of normalized MALDI-MS data of a) tributylphosphine-terminated polystyrene 2 ku series, b) Br-terminated polystyrene 2ku series ionized with silver trifluoroacetate, and c) vinyl-terminated PS 2ku series ionized with silver trifluoroacetate.

information about signal quality (i.e., signal-to-noise ratio and sensitivity), which is necessary for accurate determination of peaks in low abundance. Currently, work is in progress for such a study.

MALDI-MS analysis allows molecular mass moment determination of polymers of similar mass but different end groups. M_n comparison determined from the MALDI-MS data of the covalently cationized and silver cationized polystyrene samples indicates the following (Table 4). The intact Br-PS series give M_n values higher than the values determined for the TBP-terminated series, while the vinyl-terminated series give lower values relative to the TBP-charged series. As stated above, TBP oligomers were detected using milder laser power than the silver cationized Br-PS samples. Since the Br-PS were generated in the presence of salt and under more aggressive desorption conditions, the polymer signal was more susceptible to matrix/silver cluster ion suppression effects.^{33–35} For the Br-PS 2 ku, the intact bromine series shows a relative increase of the higher mass signal leading to a slightly higher M_n value than expected, as exemplified in Figure 10. In the vinyl case, laser-induced fragmentation dominates leading to lower M_n value than expected (Figure 10).

The M_n values of the end-functionalized polystyrene differ depending on the characterization method used for the molecular mass moment determination. As a general trend, the NMR-determined M_n values were the highest, followed by those determined by the MALDI-MS analysis. M_n values determined from the GPC data were consistently the lowest. Several factors must be

considered when explaining differences observed for the techniques including the following: (1) accurate quantitation by MS analysis of the low-mass tails of the 10–40 ku samples, (2) accurate integration of the methyl end group region for ^1H NMR analysis, and (3) accurate M_n determination biased by end group variation and limited by apparent polystyrene mass calibration for GPC analysis.^{33–37} Currently, we are investigating an approach to better evaluate uncertainties associated with the three techniques in order to make reliable comparisons.

Conclusions

We have demonstrated that the covalent cationization method can be used as a polymer ionization method over a broad selection of molar mass ranges. Br-PS of various molecular masses was synthesized by ATRP, followed by bromine substitution by TBP to form TBP-PS. GPC results of Br-PS and TBP-PS suggest that chemical modification does not affect the polydispersity or overall polymer peak shape. HPLC separation at the critical point of adsorption allows for quantitative estimation of TBP conversion in polystyrene. TBP-PS samples analyzed by MALDI-MS show similar molecular mass moments to the silver cationized Br-PS samples. Furthermore, the covalent cationization method yielded stronger and cleaner signals under milder laser conditions than samples analyzed using the traditional metal cationization. Covalent cationization targets only a single end group and would not be useful as a universal ionization method or for mixture analysis of end groups. However, the ionization technique may prove useful in improving the MALDI polymer analysis through comparative desorption and ionization studies.

The current polystyrene results suggest that the mass discrimination and peak shape distortion observed in our previous covalently cationized polyethylene study arise from the polymer behavior in matrix media and not the chemical derivatization itself. Unlike polystyrene, polyethylene forms crystalline regions in the MALDI matrix upon solvent evaporation. Currently, we are addressing the effects of polyethylene crystallinity on MALDI-MS signal.

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