MALDI/TOF/MS as a Method for Characterizing Micelle-Forming Polymers: A MALDI/TOF/MS Study of Amphiphilic Diblock Copolymers Based on Sulfonated Polystyrene

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Matrix-assisted laser desorption/ionization time-of-flight mass spectra (MALDI/TOF/MS) of sodium poly(styrenesulfonate)-block-poly (tert-butylstyrene) (NaPSS-b-PtBS) and polystyrene-block-poly (tert-butylstyrene) (PS-b-PtBS) diblock copolymers were obtained. MALDI/TOF/MS of NaPSS-b-PtBS directly yields molecular weights and polydispersities of these micelle-forming amphiphilic block copolymers. MALDI/TOF/MS allows direct determination of molecular weights and molecular weight distributions of NaPP-b-PtBS. Classical molecular weight methods, such as light scattering and osmometry, are not suitable for molecular weight determinations of these polymers due to their extremely low critical micelle concentrations. By comparing the molecular weights of NaPSS-b-PtBS and their precursors, PS-b-PtBS, extents of sulfonation were also obtained.

INTRODUCTION

Asymmetric sodium poly(styrenesulfonate)-*block*-poly(*tert*-butylstyr-ene), NaPSS-*b*-P*t*BS, diblock copolymers, with long hydrophilic blocks of NaPSS and short hydrophobic blocks of P*t*BS have been employed recently in the study of charged polymers at interfaces. This is due to adsorption of these materials at hydrophobic surfaces in aqueous media under certain conditions[1–5]. The short hydrophobic blocks act as anchors to tether the long hydrophilic blocks, which are polyelec-trolytes, to the surface. Mushroom- or brush-structured polymer layers may be formed. These materials have also been used in the study of micelles formed by charged/neutral block copolymers[6,7] in an aque-ous solution.

In the study of such amphiphilic diblock copolymers, it was found that it is difficult to characterize individual diblock copolymer chains directly due to their micelle-forming properties. It was found that the critical micelle concentration (cmc) of NaPSS-*b*-P*t*BS (4.6 wt% of P*t*BS, $M_w = 8.0 \times 10^4$) was at or below 2×10^{-4} wt% in water[8]. This is much lower than the concentrations used in traditional characterization methods, such as size exclusion chromatography, membrane osmometry, and laser light scattering. Thus, characterization data obtained on such copolymers by these traditional methods yields information on micelles, not on in-dividual chains.

Matrix-assisted laser desorption/ionization time of flight mass spec-troscopy (MALDI/TOF/MS) provides a new and useful means for characterizing polymers[9–12]. It can provide information on molecular weight, molecular weight distribution, mass of end groups, and mass of repeating units. Additional information, such as reactivity ratios, in-dividual block chain lengths and their distribution, etc., can be obtained based on the masses measured[13].

Poly(styrene sulfonic acid) and its sodium salt have been success-fully characterized by MALDI/TOF/MS[14,15], but no MALDI/TOF/TOF study of diblock copolymers containing a poly(styrene sulfonic acid) block has been reported. Herein we report the results of a MALDI/TOF/MS study of amphiphilic diblock copolymers, NaPSS-*b*-P*t*BS, and their precursors, PS-*b*-P*t*BS (PS is polystyrene and P*t*BS is poly(*tert*-butylstyrene)). Number-average and weight-average molecular weights (M_n and M_w), polydispersity indices, and extents of sulfonation were obtained. It is shown that the use of MALDI/TOF/MS in characterizing these polymers overcomes the difficulties encountered in characterizing micelle-forming polymers using tradi-tional methods.

EXPERIMENTAL SECTION

Materials

Block copolymers were synthesized using anionic polymerization according to methods described in Ref. [16]. To briefly summarize the procedures used, polymerizations were conducted at room temperature for about two days by introducing benzene (solvent), *sec*-butyllithium (initiator), *tert*-butylstyrene and styrene (monomers), and methanol (terminating agent) sequentially into evacuated, all-glass reactors. PS-*b*-P*t*BS samples were characterized by SEC and multi-angle laser light scattering, MALLS in tetrahydrofuran (THF; see Table I). These uncharged diblocks are completely soluble in THF and exhibit no tendency to micellize.

Sulfonation reactions were conducted according to the method of Valint and Bock[17]. NaPSS homopolymers studied were narrow molecular weight distribution calibration standards purchased from Pressure Chemical Company (Pittsburgh, PA).

THF used in MALDI/TOF/MS was purified by exposure to CaH$_2$ and Na/K alloy, sequentially, under high vacuum conditions. Water used in MALDI/TOF/MS was purified using a Milli-Q (Millipore) system. Other reagents for MALDI/TOF/MS (see below) were used as received.

MALDI/TOF/MS

MALDI/TOF/MS measurements were performed on a Voyager Elite DE System (PerSeptive Biosystems, Framingham, MA), operating in the linear mode. Generally, 0.1 wt% polymer solutions were prepared at least one day before use. For PS-*b*-P*t*BS analysis the solvent used was THF; for NaPSS-*b*-P*t*BS water was used as solvent. The matrix solution was prepared just prior to use. For PS-*b*-P*t*BS, the matrix solution was

TABLE I Characteristics of Polystyrene-*block*-poly(*tert*-butylstyrene)

| Sample | Polymerization degree PS/P*t*BS | SEC[a] | | | | MALLS |
		$M_n \times 10^{-4}$	$M_w \times 10^{-4}$	$M_p \times 10^{-4}$	M_w/M_n	$M_w \times 10^{-4}$
1a	114/4	1.25	1.33	1.27	1.07	1.3
2a	158/9	1.79	1.89	1.82	1.05	N/A
3a	500/17	5.47	5.73	5.59	1.05	5.7

[a] SEC was calibrated using polystyrene standards.

prepared by mixing 10 mg *trans,trans*-1,4-diphenyl-1,3-butadiene and 0.5 mL THF together and vortexing for 30 s. A trace amount of silver acetate was added to this solution. For NaPSS-*b*-P*t*BS diblock copolymers, the matrix solution was prepared by mixing 10 mg 3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid), and 0.7 mL 0.1% trifluoroacetic acid, TFA, and 0.3 mL acetonitrile together, vortexing for 1 min and centrifuging for 20 s to remove the undissolved matrix from solution. No salt was added. The polymer and matrix solutions were mixed together at polymer matrix solution volumetric ratios ranging typically from 1 to 0.01. After vortexing for 30 s, about 3 μL of polymer-matrix solution was deposited and crystallized on the sample plate by repeating the deposition-evaporation cycle three times.

NaPSS-*b*-P*t*BS diblock copolymers were also treated by cation exchange with a column of Dowex 50 W (Aldrich) ion-exchange resin. Approximately 18 mL of a 50 mL burette was filled with the resin, and approximately 100 mg of polymer in 2 mL of water was added to the column and eluted with deionized water. The sample was collected in a glass vial after the pH dropped below 6 and was used directly.

In MALDI/TOF/MS measurements, ions were formed by 337 nm laser desorption, accelerated to ±25 KV, and detected as positive or negative ions. The spectra were the sum of 128 laser shots. An external calibration using apomyoglobin and thioredoxin was used.

Fluorescence Experiments

All fluorescence measurements were made with a Perkin-Elmer Model LS 50B luminescence spectrometer. Samples (in aqueous solution) in quartz cuvettes at room temperature were illuminated with a xenon discharge lamp. The emission was collected at normal position relative to the illumination beam. The excitation and emission slits were both 5 nm. Scan speed was 250 nm/min. The excitation was at wavelength 240 nm. All spectra were the average of 5 scans. The emission spectra are uncorrected for spectral response of the analyzing system.

Elemental Analysis

All elemental analyses were performed by Atlantic Microlabs (Norcross, GA).

RESULTS AND DISCUSSION

Figures 1–3 show that PS-*b*-P*t*BS samples 1a, 2a, and 3a all exhibit symmetric peaks in their positive-ion MALDI/TOF mass spectra.
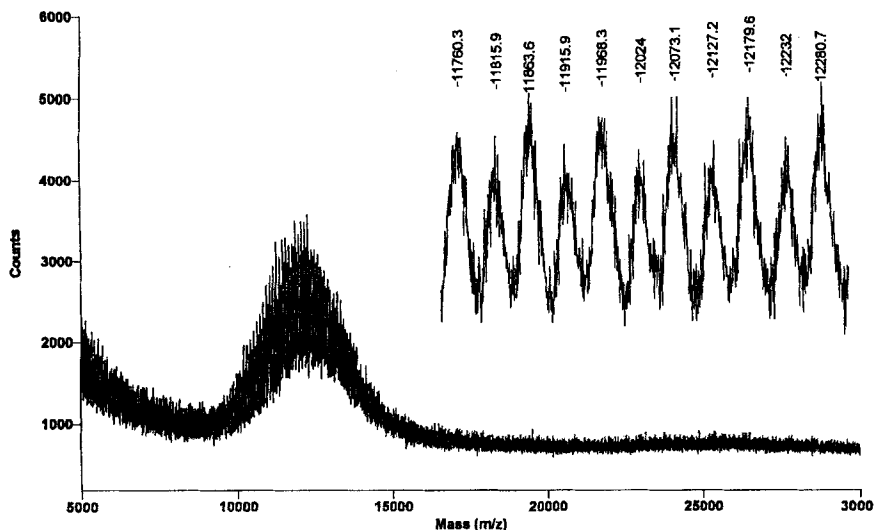
FIGURE 1 Positive-ion MALDI/TOF mass spectrum of polystyrene-*block*-poly (*tert*-butylstyrene) sample 1a. The insert is an expansion of some peaks. $M_n = 12,000$, $M_p = 12,000$, $M_w/M_n = 1.03$.
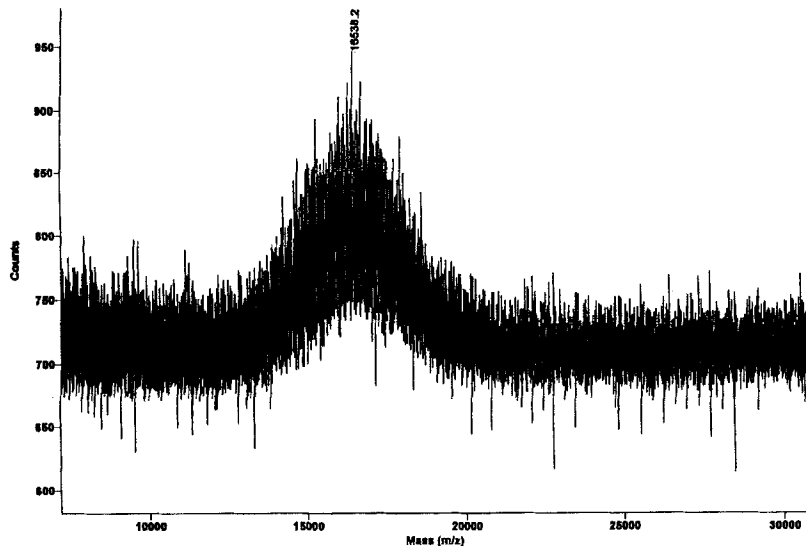

FIGURE 2 Positive-ion MALDI/TOF mass spectrum of polystyrene-*block*-poly(*tert*-butylstyrene) sample 2a. $M_n = 16,500$, $M_p = 16,500$, $M_w/M_n = 1.03$.
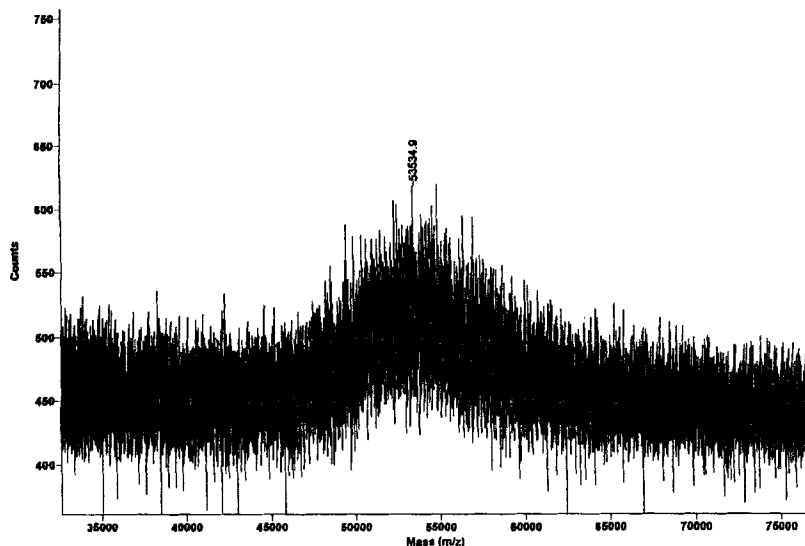
FIGURE 3 Positive-ion MALDI/TOF mass spectrum of polystyrene-*block*-poly(*tert*-butylstyrene) sample 3a. $M_n = 54,500$, $M_p = 54,500$, $M_w/M_n = 1.01$.

This is similar to the nature of their SEC curves (not shown). The M_n values obtained by MALDI/TOF/MS were the same as those obtained by SEC and MALLS within the limits of error of the latter two techniques (see Tables I and II). Polydispersity indices obtained from MALDI/TOF/MS were lower than those obtained from SEC measurements, probably due to failure to correct the SEC chromatograms for axial dispersion.

For sample 1a (Figure 1), oligomer resolution was achieved in the mass spectrum. On close examination it can be seen that each peak is a cluster of many peaks, which have masses very close to each other (see insert). This reflects the diblock nature of these materials. There are two series of peaks that appear in the spectrum alternatively. Peaks in one series have higher intensities than the peaks in the other series. The mass increment within each series is 104.45 (± 0.03; $n = 24$) g/mol, which is close to the mass of the polystyrene repeating unit. This is reasonable since this diblock copolymer is 95 wt% PS. The mass difference between two adjacent peaks (which belong to different series) is about 56 g/mol, which is the mass difference between repeating units of P*t*BS and PS. Due to the limited resolution within one peak, a more detailed analysis cannot be carried out.

The negative-ion mass spectra of NaPSS-*b*-P*t*BS diblock copolymers 1b, 2b, and 3b, which were prepared from PS-*b*-P*t*BS diblock copolymers

TABLE II MALDI/TOF/MS Characterization of NaPSS-b-PtBS and PS-b-PtBS

Sample[a]	$M_n \times 10^{-4}$	$M_w \times 10^{-4}$	M_w/M_n	$M_p \times 10^{-4}$	Sulfonation degree (%)		
					MALDI[b]	MALDI[c]	Elemental analysis[d]
1a	1.20	1.24	1.03	1.20			
1b	1.79	1.83	1.02	1.80	82	86.3	91.5
2a	1.65	1.70	1.03	1.65			
2b	2.80	2.97	1.06	2.43	99	85.6	87.5
3a	5.45	5.50	1.01	5.45			
3b	9.37	9.74	1.04	9.56	99	101	86.5

[a] Samples 1b, 2b, and 3b are PSSA-b-PtBS prepared from samples 1a, 2a, and 3a of PS-b-PtBS, respectively.
[b] Based on the M_n in MALDI/TOF/MS.
[c] Based on the M_p in MALDI/TOF/MS.
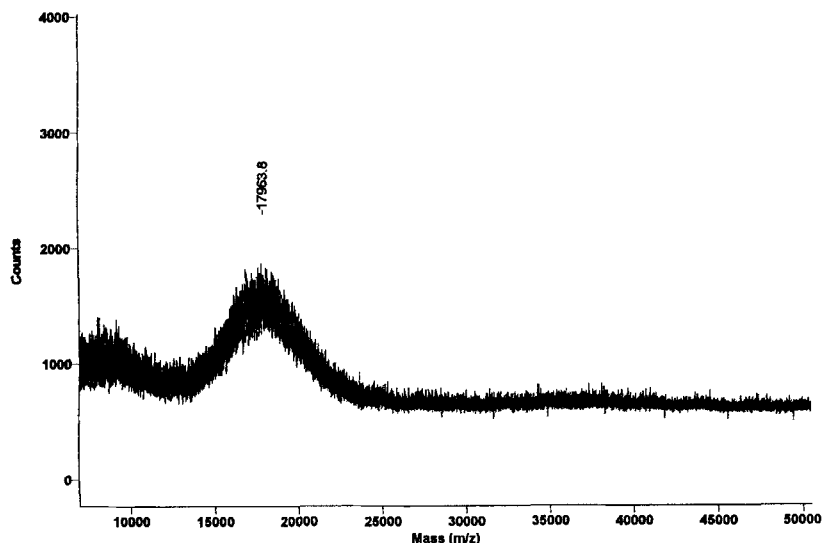[d] Based on elemental analysis for sulfur.

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FIGURE 4 Negative-ion MALDI/TOF mass spectrum of sodium poly(styrene-sulfonate)-*block*-poly(*tert*-butylstyrene) sample 1b. $M_n = 17,900$, $M_p = 18,000$, $M_w/M_n = 1.02$.

1a, 2a, and 3a, respectively, by sulfonation are shown in Figures 4–6. Before discussion of these mass spectra, it is important to note that MALDI/TOF/MS experiments on these polymeric amphiphiles were performed at concentrations higher than the cmc. The cmc of sample 3b was estimated in our lab via the fluorescence spectrometry method used by Guenoun et al.[8] Figures 7, 8, and 9 show the emission spectra of homopolymer (NaPSS) and diblock copolymer (NaPSS-*b*-P*t*BS) sample 3b in aqueous solution at different concentrations ranging from 1×10^{-7} to 1×10^{-4} g/g. By comparison of the respective monomeric and excimeric emission peaks of about 290 nm and 330 nm of both diblock copolymer and homopolymer, the cmc of sample 3b was estimated at less than 1×10^{-6} g/g. These results are in agreement with those obtained in Ref. [8], in which a P*t*BS/NaPSS diblock copolymer with similar composition was studied.

The lowest polymer concentration employed in MALDI/TOF/MS measurements was around 1×10^{-5} g/g, which is higher than the cmc of sample 3b. It is therefore clear that MALDI/TOF/MS is a useful tool in the study of amphiphilic diblock copolymers, even when they have cmc values lower than concentrations used in MALDI/TOF/MS. No signal corresponding to the molecular weight of the micelles could be detected in these experiments. It thus seems likely that the laser excitation process
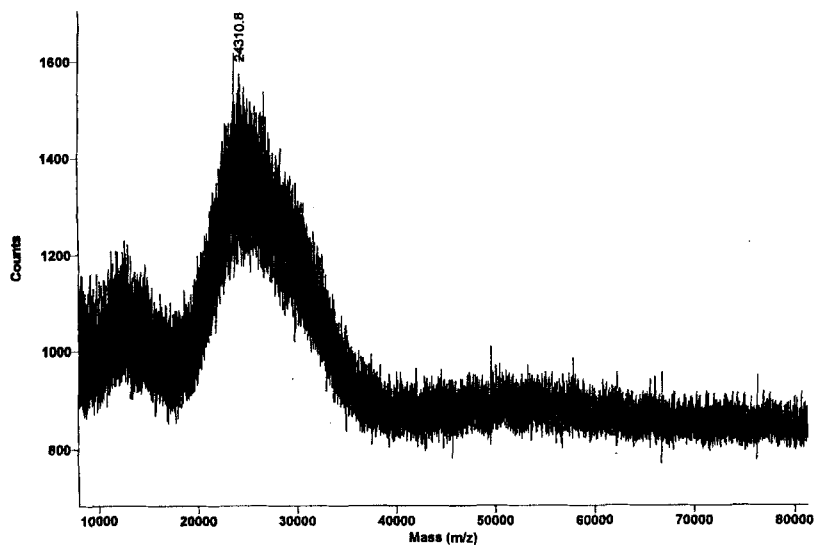
FIGURE 5 Negative-ion MALDI/TOF mass spectrum of sodium poly(styrene-sulfonate)-*block*-poly(*tert*-butylstyrene) sample 2b. $M_n = 28,000$, $M_p = 24,300$, $M_w/M_n = 1.06$.
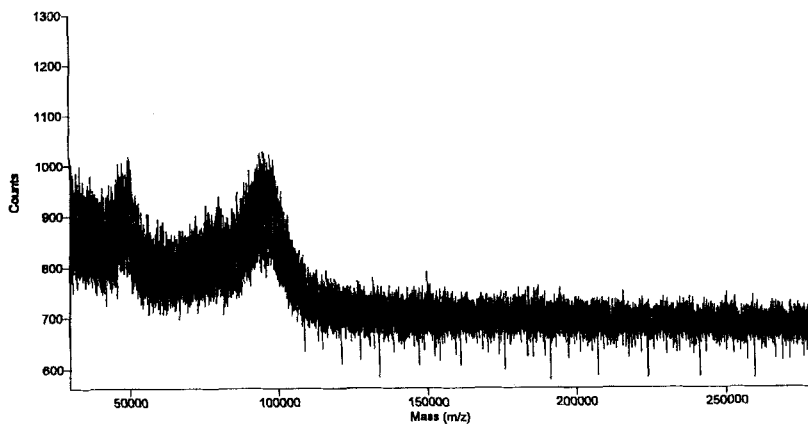

FIGURE 6 Negative-ion MALDI/TOF mass spectrum of sodium poly(styrene-sulfonate)-*block*-poly(*tert*-butylstyrene) sample 3b. $M_n = 93,700$, $M_p = 95,600$, $M_w/M_n = 1.04$.
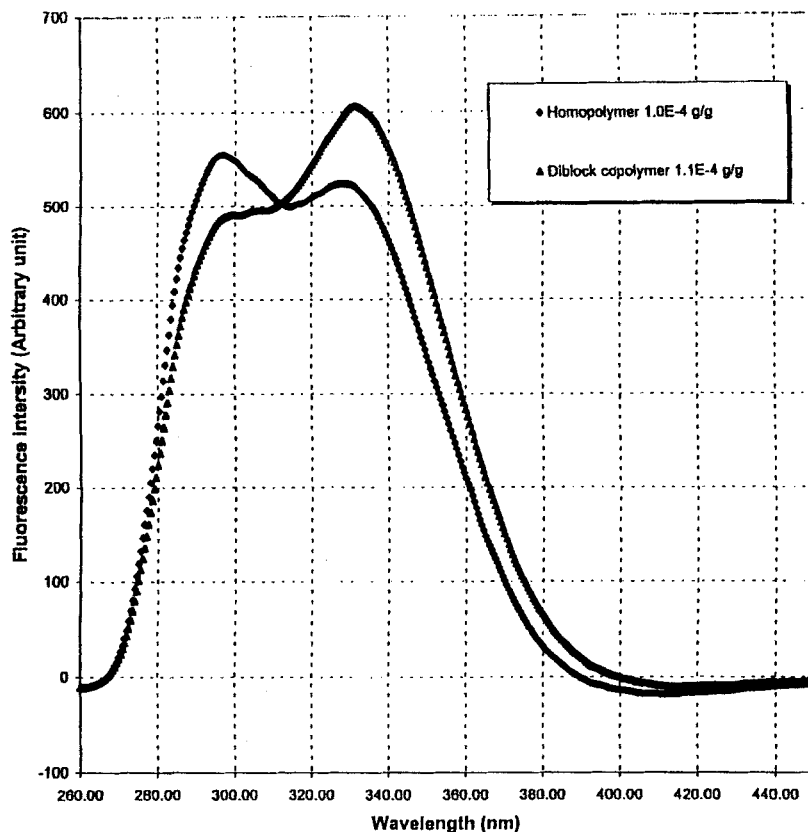
FIGURE 7 Comparison of fluorescence emission spectra for NaPSS homopolymer ($M_w = 74,000$) and sample 3b, NaPSS-b-PtBS, $M_w = 97,400$, diblock copolymer in water at 1×10^{-4} g/g.

used in obtaining a MALDI spectrum is energetic enough to cause micelles present in the matrix crystals to "break down" into individual polymer chains (unimers). However, our prior and ongoing studies of micelles of these materials indicate that a low level of unimers exist in equilibrium with the micelles. These unimers constitute a second potential source of the individual chains detected in MALDI/TOF/MS.

Positive-ion mass spectra of samples 1b and 2b were also obtained (see Figures 10 and 11) by changing the detection to positive ion mode while all other instrumental conditions were kept the same. The mass spectra (their shapes and mass-to-charge (m/z) positions) obtained in negative and positive ion modes were essentially the same. This result is different
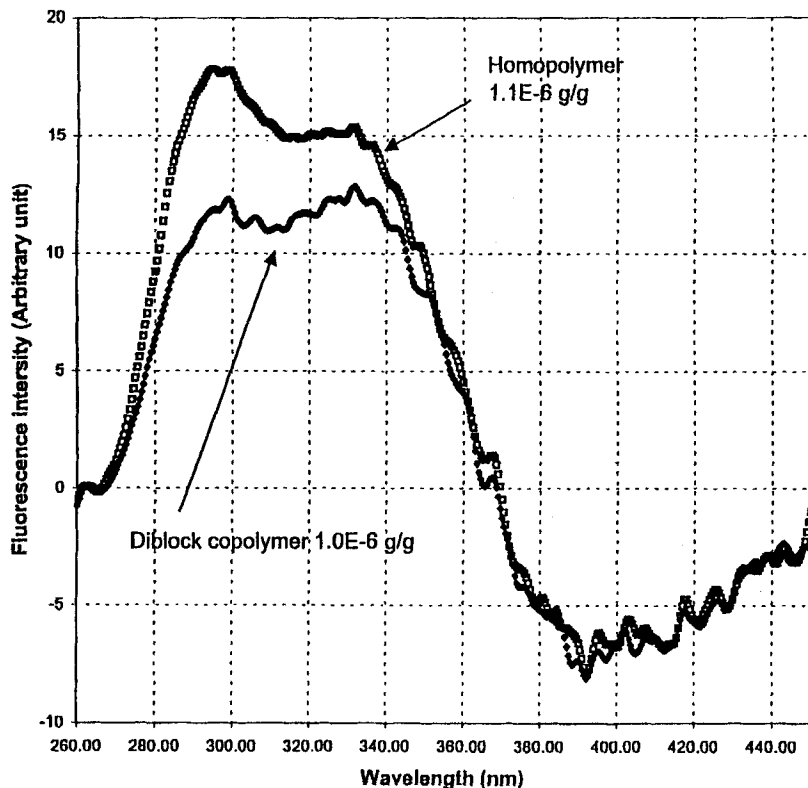
FIGURE 8 Comparison of fluorescence emission spectra for NaPSS homopolymer and NaPSS-*b*-P*t*BS sample 3b at 1×10^{-6} g/g.

from results obtained by Danis et al.[14] and Rader et al.[15] for NaPSS homopolymers. They found that positive-ion mass spectra of NaPSS exhibited higher masses than the corresponding negative-ion mass spectra. Such a discrepancy is not surprising for sample 1b because elemental analysis (Table III) revealed that there was only a limited amount of sodium in this sample. The sodium content was 1.7% in the total mass, which corresponds to about only 16% of its theoretical value. However, for sample 2b, our results were surprising because elemental analysis revealed that this sample had 10.6 wt% sodium, which corresponds to about 99% of the theoretical content for the pure monosodium salt.

To clarify this discrepancy, we used a cation-exchange resin to remove metal ions from the polymer samples. After cation exchange, positive-ion mass spectra of both samples 1b and 2b still exhibited the same m/z (see Figures 12 and 13). This observation suggests that under our
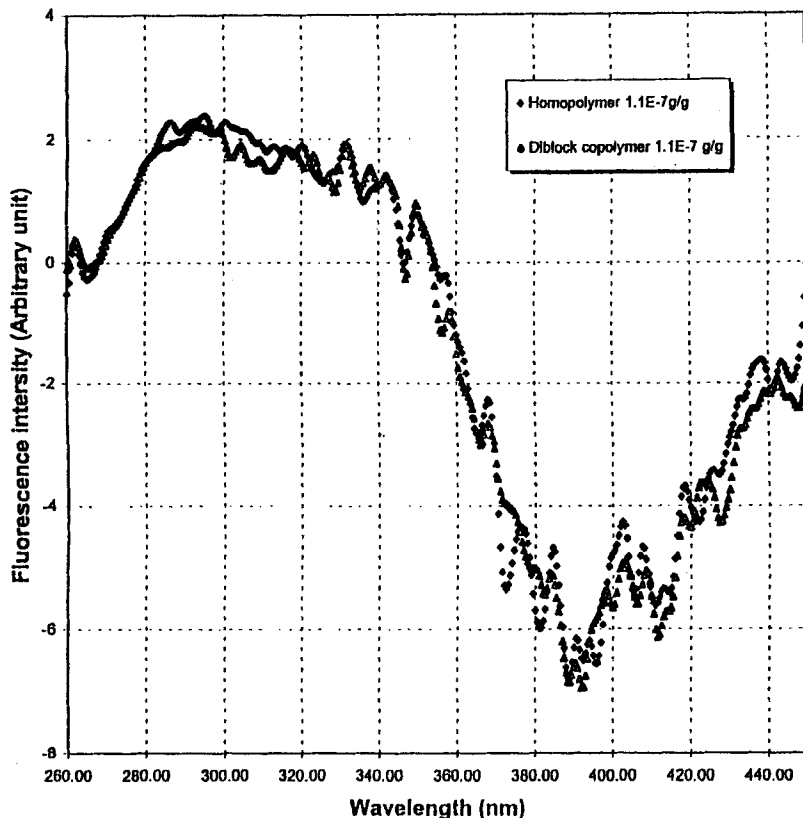
FIGURE 9 Comparison of fluorescence emission spectra for NaPSS homo-polymer and block copolymer sample 3b at concentration 1×10^{-7} g/g.

experimental conditions the acid form of poly(styrene sulfonic acid) was analyzed, whereas Danis et al.[14] and Rader et al.[15] analyzed the salt form. Differences in sample preparation may be important. In our MALDI/TOF/MS experiments, the matrix used was sinapinic acid and it was dissolved in an aqueous solution of trifluoroacetic acid and acet-onitrile (0.7 mL 0.1% trifluoroacetic acid and 0.3 mL acetonitrile). In the work by Danis et al.[14], although sinapinic acid was employed as a matrix without the use of trifluoroacetic acid and acetonitrile. In the study by Rader et al.[15], both 2,5-dihydroxybenzoic acid and sinapinic acid were used as matrices. No addition of trifluoroacetic acid or acetonitrile was mentioned. Given the strongly acidic nature of trifluoroacetic acid, our observations (positive-ion and negative-ion mass spectra were the same) might be expected.
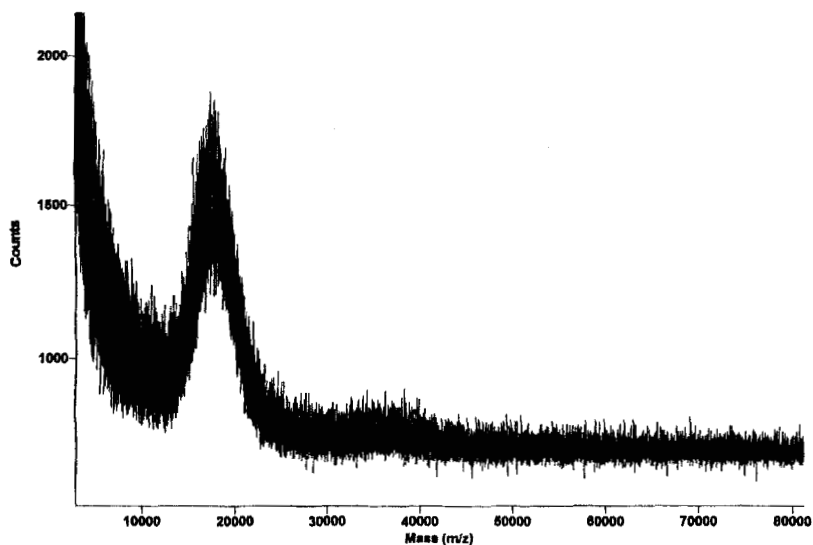
FIGURE 10 Positive-ion MALDI/TOF mass spectrum of sodium poly(styrene-sulfonate)-*block*-poly(*tert*-butylstyrene) sample 1b.
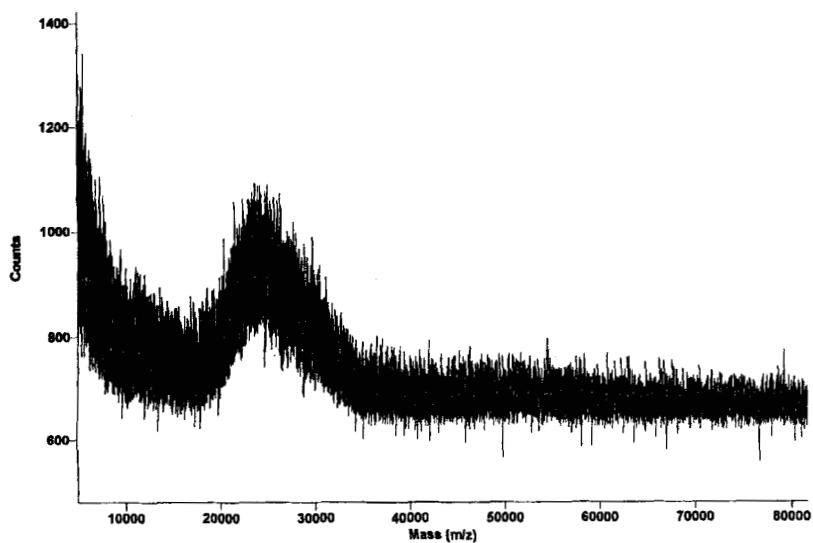

FIGURE 11 Positive-ion MALDI/TOF mass spectrum of sodium poly(styrene-sulfonate)-*block*-poly(*tert*-butylstyrene) sample 2b.

TABLE III Elemental Analysis Results for NaPSS-*b*-P*t*BS Samples

Sample	Element	Theory[a] wt%	Found wt%
1b	S	15.2	14.2
	Na	10.9	1.7
2b	S	14.9	13.0
	Na	10.7	10.6
3b	S	15.2	13.2

[a] These data were calculated from composition data of each sample and the assumption that all polystyrene blocks were converted to sodium poly(styrenesulfonate) and all poly(*tert*-butylstyrene) blocks were unaffected by sulfonation.

Dimer peaks in MALDI/TOF mass spectra are often observed[18], since dimers may be formed during laser desorption. However, with our samples dimerization could also occur during the sulfonation reaction due to sulfone formation. For sample 2b, there is a small dimer peak at m/z 5,100. This dimer peak was still found when the laser power was increased to 170% of the threshold laser power value. This suggests that there may be some small amount of sulfone formation, resulting in dimerization, during the sulfonation reaction to synthesize sample 2b.

Sample 1b exhibited a symmetric peak in its negative-ion mass spectrum and polydispersity indices of samples 1a and 1b, obtained by MALDI/TOF/MS, are essentially the same. This suggests that the
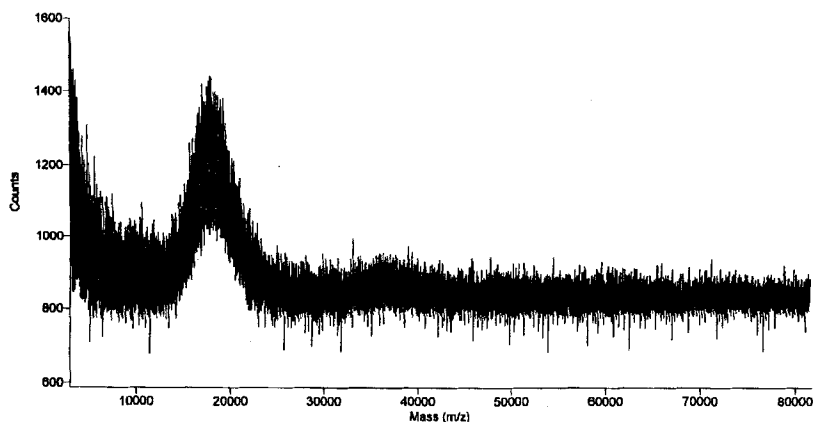

FIGURE 12 Positive-ion MALDI/TOF mass spectrum of sodium poly(styrenesulfonate)-*block*-poly(*tert*-butylstyrene) sample 1b after ion-exchange resin treatment.

FIGURE 13 Positive-ion MALDI/TOF mass spectrum of sodium poly(styrene-sulfonate)-*block*-poly(*tert*-butylstyrene) sample 2b after ion-exchange resin treatment.
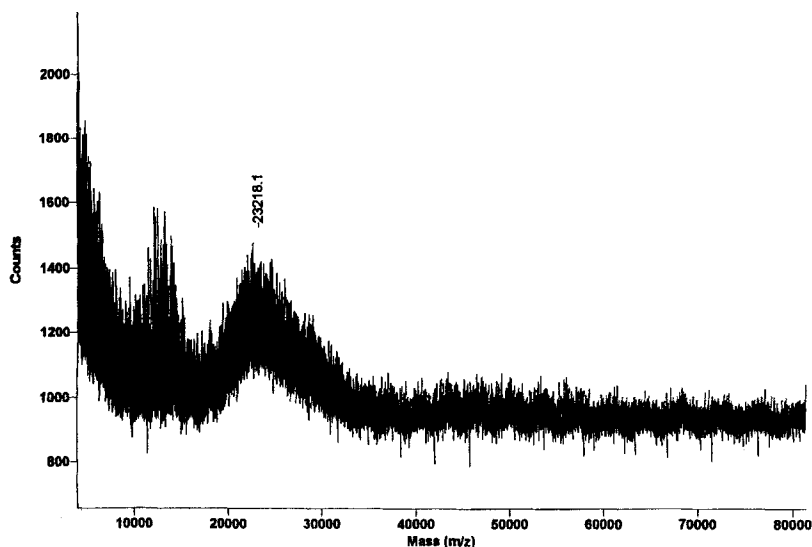
sulfonation level was uniform (or nearly so) for all polymer chains in this sample across the entire mass distribution. However, samples 2b and 3b exhibited asymmetric peaks in their negative-ion mass spectra. Although their polydispersity indices are still small (1.06 and 1.04 for samples 2b and 3b, respectively), they are larger than the polydispersity indices of samples 2a and 3a obtained by MALDI/TOF/MS. This suggests that the sulfonation level is not uniform across the molecular weight distribution for these samples. Such mass distribution curves (actually mass/charge ratio curves) further demonstrate the advantages of MALDI/TOF/MS in characterizing such micelle-forming polymers.

Table II shows peak maximum mass (M_p), M_n, M_w, polydispersity index (M_w/M_n), and the sulfonation degrees of all samples, as obtained from MALDI/TOF/MS. By comparing the molecular weights of each pair of PS-*b*-P*t*BS and NaPSS-*b*-P*t*BS, combined with the knowledge of compositions of each PS-*b*-P*t*BS diblock copolymers, the sulfonation degree can be calculated according to

$$\text{Sulfonation Degree}(\%) = \frac{M_2 - M_1 \cdot PtBSwt\%}{M_1 \cdot PSwt\% \cdot (184/104)} \times 100\%$$

in which M_1 is the molecular weight (M_p or M_n) of the unsulfonated sample in MALDI/TOF/MS and M_2 is the molecular weight of the

sulfonated sample. The values 184 and 104 correspond to the masses of repeating units of poly(styrene sulfonic acid) and polystyrene.

The sulfonation degrees were also obtained from elemental analysis for sulfur (see Table III). For sample 1b, the sulfonation degrees obtained from MALDI and elemental analysis are close to each other. For samples 2b and 3b, the sulfonation degrees obtained from MALDI are different from those obtained from elemental analysis. This might be due to the asymmetric shapes of the mass spectra of samples 2b and 3b. It should also be noted that the elemental analytical results on extent of sulfonation are susceptible to errors if low molecular weight sulfur-containing impurities (byproducts of the sulfonation reaction) are present.

CONCLUSIONS

MALDI/TOF mass spectra of individual amphiphilic diblock copolymers, NaPSS-*b*-P*t*BS, as well as their precursors, PS-*b*-P*t*BS, were obtained. Their molecular weights and polydispersities can be calculated directly from the mass spectra. No other experimental technique (e.g, light scattering and osmometry) allows direct molecular weight determination of these polymeric amphiphiles due to their very low critical micelle concentrations. The extents of sulfonation were calculated by comparing the molecular weights of each pair of PS-*b*-P*t*BS and NaPSS-*b*-P*t*BS copolymers. MALDI/TOF/MS provides a useful method for characterizing micelle-forming block copolymers.

ACKNOWLEDGMENTS

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