Analysis of Poly(styrenesulfonic acid) by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry

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ABSTRACT: Matrix-assisted laser desorption/ionization time-of-flight (MALDI/TOF) mass spectrometry has been used for the analysis of poly(styrenesulfonic acid), and the molecular weight distributions have been determined for polymers up to 430 000 Da in mass. Poly(styrenesulfonic acid) and the sodium salt of the poly(styrenesulfonate) were analyzed by MALDI/TOF with detection of negative or positive ions, respectively. The singly charged and several multiply charged distributions were observed for polymers of average molecular weight 18 000, 100 000, and 400 000 Da. Agreement with the manufacturer's values for average molecular weight is good with the exception of the 18 000 Da sample which MALDI/TOF indicated was only partially sulfonated. This was confirmed by elemental analysis.

Introduction

The relatively new technique of matrix-assisted laser desorption/ionization time-of-flight (MALDI/TOF) 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 polysaccharrides.[10,11] Recent results[12−20] have also shown its use as an effective polymer molecular weight tool. MALDI/TOF generally produces molecular ions without fragmentation[1−4] and has no reliance on polymer standards or Mark−Houwink constants[21] for molecular weight assignments; the distribution and average molecular weight are obtained simultaneously. Molecular weights on narrow distribution polymers have been determined with an accuracy as good as light scattering, osmometry, and viscometry,[14,15,20] and polymer distributions and polydispersity indices from MALDI/TOF agree well with those from GPC.[13−15,18] Previous work has shown that synthetic polymers of up to 200 000 Da can be measured with this technique.[13] Here we are extending the capability of MALDI/TOF to provide molecular weight distributions of polymers up to 430 000 Da.

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. All measurements shown were made in the linear mode with the three-stage detector (due to the increased sensitivity). The detector consists of a discrete dynode followed by a single microchannel plate. Following the microchannel plate there is variable acceleration (0−15 kV; 7 kV was used for these experiments) of the electrons into a scintillator, and the resulting photons are detected with a photomultiplier tube. Samples were prepared in a sinapinic acid (Aldrich, Milwaukee, WI) matrix at a polymer:matrix mole ratio of 10^{-3} with a total loading of polymer around 100 pmol (this is based on the manufacturer's value for the average molecular weight). Ions were formed by laser desorption at 337 nm (LSI N_2 laser, 3 ns pulse width, 10^6 to 10^7 W/cm^2, 100 μm diameter spot) and accelerated with 25−35 kV.

The poly(styrenesulfonate) samples (peak-average molecular weight,[22] M_p = 18 000, 100 000, and 400 000 Da) are from Polymer Laboratories (Amherst, MA) and are supplied as the sodium salt of the acids. Since salts have been shown to have a deleterious effect on MALDI,[6] the polymers were also prepared in the acid form by ion exchange with a column of Amberlite IRN-77 (Rohm and Haas Co., Philadelphia, PA) ion-exchange resin. Approximately 20 mL of a 50 mL buret 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 polypropylene vial when the pH dropped below 4 and then freeze dried. For long term storage the polymer was left in solution since the solid poly(styrenesulfonic acid) is unstable at room temperature for periods longer than 1 month.

Negative ion spectra were acquired on the poly(styrenesulfonic acids) and positive ion spectra on the original sodium salts. All mass spectra shown were baseline corrected and smoothed by the Sun SPARCstation LX data system running the Bruker XMASS software. The baseline was corrected by subtracting the matrix background which was fit by a decreasing exponential function. After baseline correction, an eleven point Golay−Sawitski smooth was applied. All average molecular weight calculations were made by the data system using Bruker PolyMass software. The calculations were based on the integration of the peak areas at an interval of m/z 200 and follow standard calculations of M_w and M_n.[21]

Gel permeation chromatography was performed on Progel TSK columns (Supelco, Bellefonte, PA) connected in series: G4000SWXI (7.8 × 300 mm; 8 μm particle size) and G3000SWXL (7.8 × 300 mg; 8 μm particle size). The mobile phase was 0.05 M sodium acetate at a flow rate of 1.0 mL/min using a Waters 6000A pump (Milford, MA). Injections of 50 mL (1 mg/mL) were made on a Valco six-port valve (Houston, TX), and detection was via a Waters 410 refractive index detector. All measurements were made at ambient temperature. Data were collected on a Macintosh IIcx (San Jose, CA) with 12 bit A/D interface software from Strawberry Tree and converted to files compatible with Kaleidagraph graphical software. Elemental analysis was performed by Oneida Research Laboratories (Oneida, NY). Before analysis, 30 mg of the sample was ultrafiltered three times through an Amicon (Beverly, MA) model 8010 stirred cell using a YM1 filter (nominal MW cutoff 1000 Da) to remove any residual sodium sulfate. Eighty percent recovery was achieved.

Results and Discussion

The negative ion MALDI/TOF mass spectrum of the 100 000 Da poly(styrenesulfonic acid) is shown in Figure 1. The peak at m/z 85 000 is the polymer distribution due to the singly charged molecular ions, the doubly charged distribution is observed at m/z 43 000, and at m/z 29 000 is the triply charged distribution. This

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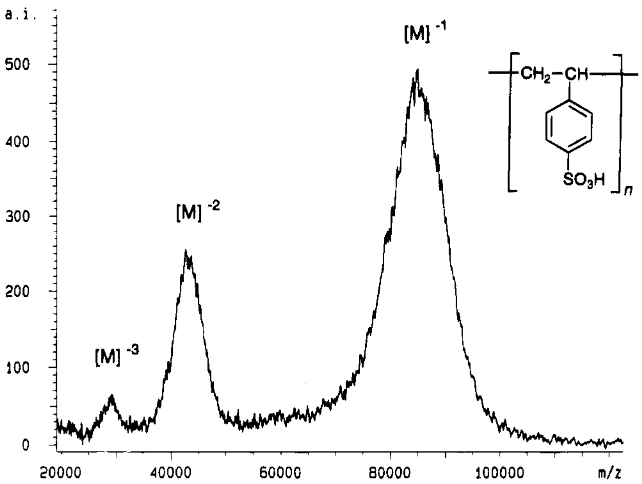

Figure 1. Negative ion MALDI/TOF mass spectrum of poly(styrenesulfonic acid) 100 000 Da.

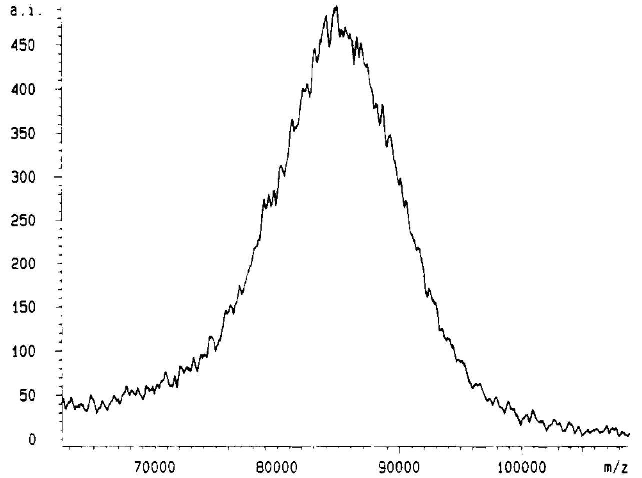

Figure 2. Negative ion MALDI/TOF mass spectrum of poly(styrenesulfonic acid) showing the singly charged ion region of Figure 1.

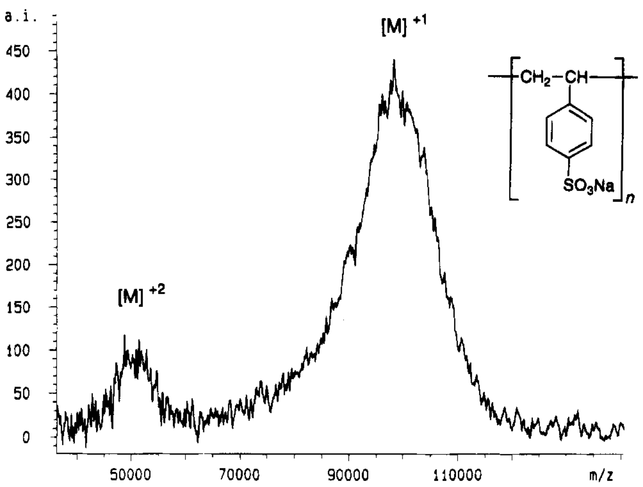

Figure 3. Positive ion MALDI/TOF mass spectrum of poly(styrenesulfonate) 100 000 Da.

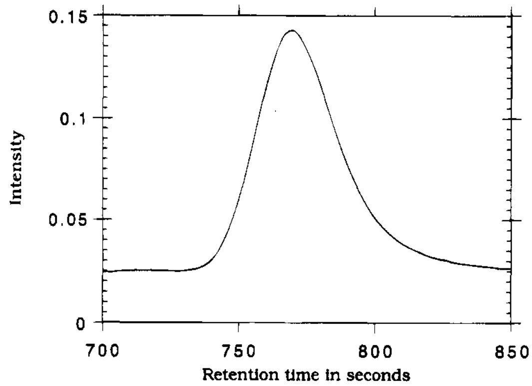

Figure 4. Gel permeation chromatogram of poly(styrenesulfonate) 100 000 Da.

multiple charging is common for MALDI and is often observed for proteins.[1-5] There is not sufficient resolution at this mass to resolve the individual molecular ions; thus, the molecular ions convolute and become a continuous distribution. It should be noted that no m/z shifts in the molecular ion clusters were observed when experimental parameters such as laser power, accelerating voltage, and matrix were varied. An expansion of the singly charged region is seen in Figure 2. The broadness of the molecular ion region is low; however, since the supplier specification quotes a polydispersity of less than 1.10, the narrow distribution of the species is expected. The numbers we obtain for this distribution are M_n = 83 900, M_w = 84 400, and M_p = 84 900 Da for a polydispersity of 1.01. Since we are measuring the sulfonic acid and not the original sodium sulfonate form of the polymer, our molecular weight distribution should be multiplied by 206/184 (monomer mass ratio for the salt and acid forms) to compare with the supplier's value. In this case our adjusted value of M_p = 95 100 Da corroborates the supplier's number.

One assumption made in these measurements is that the MALDI/TOF instrument response is constant over the mass range of the polymer distribution. This is supported by recent work[23] which has shown that a 1:1 molar mixture of oligomers of methyl methacrylate with masses of 2500 and 5000 Da gave equal responses in the MALDI/TOF mass spectrometer. Additionally, the MALDI/TOF measurement of a mixture of poly(methyl methacrylate) low-polydispersity standards of average molecular weights of 6000 and 59 000 Da has also shown nearly constant response as a function of mass.[24] Since the mass distributions of the poly(styrenesulfonic acids) measured here cover relatively narrow mass ranges, i.e. low polydispersities, the effect of any falloff in detector sensitivity is minimized. Although the relative detector response at the higher masses has not been directly measured, the close agreement between MALDI/TOF and LALLS M_w values[14,20] further suggests that the effect of detector bias is minimal.

Despite the possible problems associated with the analysis of the sodium salt, we obtained the spectrum shown in Figure 3 for the sodium salt of poly(styrenesulfonate). The distribution is similar to the negative ion spectrum, and the M_p = 98 000 Da is consistent with the manufacturer's number and the negative ion result. The signal-to-noise ratio in the positive ion spectrum is clearly less than the negative case, however; so the sodium salts do seem to have a deleterious effect. For comparison to the mass spectra, the gel permeation chromatogram is shown in Figure 4. No mass numbers are given on the x-axis because these materials are used as GPC standards for mass calibration. The GPC does show a distribution for the polymer that is similar in shape to that obtained by mass spectrometry and is further confirmation of the accuracy of the mass spectral data.

The negative ion spectrum for the 400 000 Da poly(styrenesulfonic acid) is shown in Figure 5. One observes the singly charged molecular ion region around

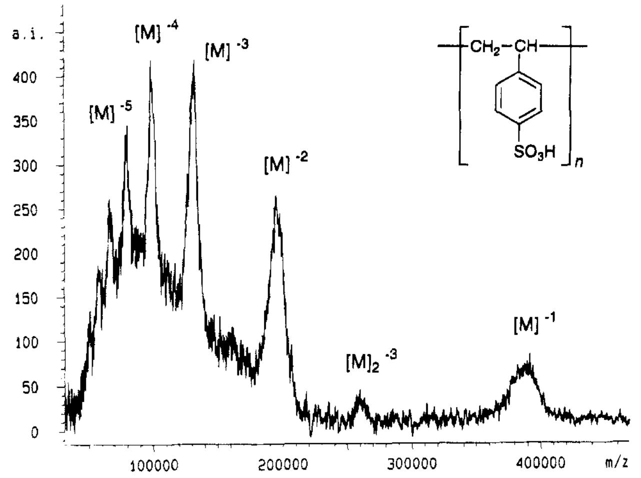

Figure 5. Negative ion MALDI/TOF mass spectrum of poly(styrenesulfonic acid) 400 000 Da.

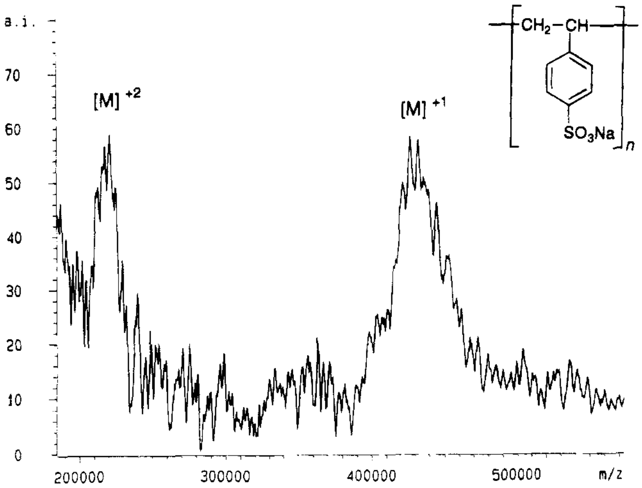

Figure 6. Positive ion MALDI/TOF mass spectrum of poly(styrenesulfonate) 400 000 Da.

m/z 388 000 and the doubly charged distribution about *m/z* 195 000 as well as the higher charge states −3 through −7 at lower masses. In addition, a peak corresponding to the triply charged dimer appears at *m/z* 260 000, showing that species of mass up to 800 000 Da can be detected with MALDI/TOF, albeit in this case with three charges on them. This clustering of the molecules is also a common process for MALDI. The M_n, M_w, and M_p we obtain for this polymer are 387 000, 388 000, and 388 000 Da, respectively, yielding a polydispersity of 1.003. This is in good agreement with the manufacturer's value of 400 000 Da (after multiplying to obtain the molecular weight for the sodium salt we obtain an M_p = 434 000 Da) and M_w/M_n < 1.10. These numbers can be further refined by including the higher charge states, as is common for electrospray ionization.[25,26] Using the doubly charged distribution, the values for M_n and M_w obtained are 194 800 and 195 000 Da, which are doubled to give 389 600 and 390 000 Da, respectively. The triply charged region yields 130 500 Da for both M_n and Mw, which is tripled to give 391 500 Da, thus showing the consistency of the spectra. We were also able to obtain a spectrum of the sodium salt of this polymer, as shown in Figure 6. The peak maximum appears about *m/z* 430 000 with a doubly charged peak at *m/z* 215 000 corroborating the negative ion result as well as the ion-exchange process to convert the polymer to the sulfonic acid. The signal-to-noise ratio is worse for the sodium salt, as was the case for the 100 000 Da poly(styrenesulfonate).

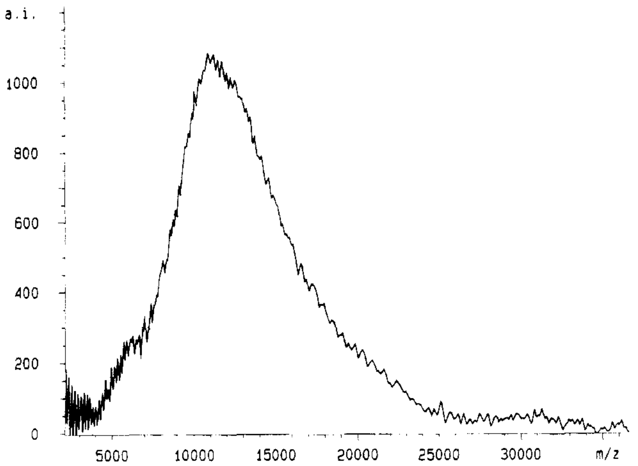

Figure 7. Negative ion MALDI/TOF mass spectrum of poly(styrenesulfonic acid) 18 000 Da.

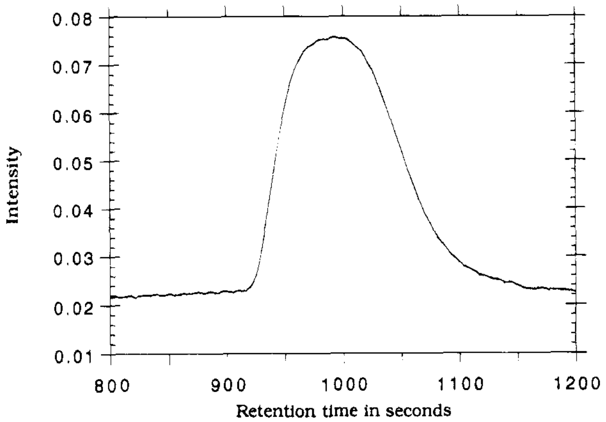

Figure 8. Gel permeation chromatogram of poly(styrenesulfonate) 18 000 Da.

An interesting case is the M_p = 18 000 Da poly(styrenesulfonic acid) spectrum in Figure 7. This profile clearly shows a defect in the distribution of the polymer and has a lower than expected molecular weight. The M_n, M_w, and M_p obtained are 12 300, 13 800, and 11 000 Da, respectively (M_p corrected to 12 300 Da for the sodium salt), giving a polydispersity of 1.12, which is much higher than the other standards measured. The manufacturer claims an M_p = 18 000 Da with a M_w/M_n < 1.10. The GPC, shown in Figure 8, has a much broader distribution with some tailing at higher retention times when compared to the GPC in Figure 4. Since GPC is a size exclusion measurement, this would imply that tailing is occurring at the low M_w end. However, this same effect can be observed when adsorption of analytes occurs on the column.[21] Elemental analysis of this sample yields a carbon/sulfur ratio of 9 in comparison to the value of 17 for a fully monosulfonated polystyrene. Thus, the sulfonation reaction did not go to completion, and this standard is a copolymer of styrene and styrenesulfonate. It has hydrophobic units which interact with the GPC stationary phase causing peak broadening, and it has a molecular weight that is significantly lower than the expected value. The mass spectrum confirms this result; the bulk of the distribution is on the low molecular weight side with the tail extending to *m/z* 25 000, where one would expect the sulfonated polystyrene to occur. This example demonstrates the utility of MALDI/TOF for examining the polymer molecular weight distribution, particularly when they are used as standards or other benchmarks.

Though, as was mentioned above, polydispersity indices from MALDI/TOF agree well with those from GPC,[13-15,18] values determined by MALDI/TOF can also sometimes be lower,[20] as in the cases here. The two explanations are that the GPC value is too high and/or the MALDI/TOF value too low. Previous work[20] discussed how the lack of band-broadening correction of the GPC can cause the value to be 5–10% above the true value.[21,27] Alternatively, a low MALDI/TOF value can be caused by a poor dynamic range or detector falloff with mass. Though the good agreement on M_w and M_n values from MALDI and conventional techniques seems to indicate that the latter two effects are not large, measurements to directly address these issues are underway in our laboratory.

Conclusions

As has been demonstrated with these polymers of styrenesulfonic acid, analysis of synthetic organic polymers by MALDI/TOF mass spectrometry provides the molecular weight as well as the distribution of the polymer. Agreement with values given by the manufacturer is generally good with the exceptions of the 18 000 Da sample which MALDI/TOF showed, and elemental analysis confirmed, to be deficient in sulfonation. The current mass range for synthetic polymers has been extended to over 400 000 Da, and this will allow for the application of MALDI/TOF to a significant number of problems in polymer science. MALDI/TOF will be able to enhance the current molecular weight techniques by measuring the molecular weight distributions of new polymer standards. Additionally, MALDI/TOF can provide the molecular weight distributions for new materials such as copolymers and grafted systems for which standards are not available. As these measurements become more facile, rapid feedback could be given to polymerization processes, resulting in greater understanding of polymer kinetics and better control of average molecular weight.

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