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Calibration for determining monomer ratios in copolymers by electrospray ionization mass spectrometry

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Abstract

Monomer ions appear in electrospray mass spectra of poly(styrene sulfonate-co-maleic acid) as the cone voltage (CV) is changed from −20 to −100 V. For a 1:1 styrene sulfonate (SS): maleic acid (MA) copolymer at CV = −100 V, the SS:MA monomer ion intensity ratio $r_{1:1} \equiv (i_{m/z\,183}/i_{m/z\,115})_{1:1}$ is 18.50 ± 0.64, reflecting the relatively high acidity of SS (pK_a 0.7, versus 1.92 for MA). When sampling a 3:1 SS:MA copolymer, $r_{3:1}$ is 84.64 ± 1.83. The ratio of these ratios $(r_{3:1}/r_{1:1})_{\text{polymer}}$ is 4.57 ± 0.18, significantly higher than the value expected [(3:1)/(1:1) = 3] based on the relative (nominal) monomer ratios in the copolymers. This compromises the use of one polymer sample as a standard for assessing the monomer ratio in the other *at this CV*. Use of monomer mixtures as standards is also precluded at CV = −100 V due to extensive fragmentation when sampling free MA monomers. Use of a lower CV and consideration of fragments as well as the monomer ions leads to better performance. At CV = −40 V, total polymer ion intensity ratios ($\Sigma \equiv \frac{i_{m/z183}+i_{m/z119}+i_{m/z80}}{i_{m/z115}+i_{m/z71}}$) are 42.60 ± 2.01 and 13.11 ± 0.87 for the 3:1 and 1:1 copolymers, respectively. The ratio of these ratios $(\Sigma_{3:1}/\Sigma_{1:1})_{\text{polymer}}$ is 3.25 ± 0.29, in good agreement with the nominal composition ratio. Similar results were obtained at CV −40 to −90 V. Using monomer mixtures as calibrants at CV = −40 V also provides accurate quantitation of the copolymer monomer ratios, provided that the total intensity ratios are used. Comparison of monomer and polymer breakdown diagrams provides the insight needed to determine the appropriate CV for quantitation with either polymer or monomer standards. Similar results are obtained for a second ionic copolymer system (2-acrylamido-2-methylpropane sulfonic acid-co-acrylic acid). Once optimum conditions are achieved, monomer ratio determination can proceed very quickly, suggesting potential utility for routine quality monitoring.

Keywords: Electrospray; Monomer ratio; Copolymer

1. Introduction

Work in our laboratory has sought to assess and extend the utility of mass spectrometry [1–17] as a faster alternative to conventional means [18–22] of characterizing the composition of copolymers. For example, we recently utilized in-source fragmentation with negative ion electrospray mass spectrometry to assess monomer ratios in copolymers of three acidic monomers (styrene sulfonic acid [SS], acrylic acid [AA], and 2-acrylamido-2-methylpropane sulfonic acid [AMPS]) [12]. In the absence of independent information about the terpolymer samples studied, extensive and time-consuming nuclear magnetic resonance (NMR) measurements were used to calibrate the ratios of monomer ions. By contrast, the monomer ratios could be assessed relatively quickly and easily by in-source depolymerization. Relative MS sensitivities to constituent monomers generally correlated with expectations based on the relative monomer acidities, suggesting that it might be feasible to use simple monomer mixtures to facilitate quantitation. We now pursue this possibility using two copolymers of SS and maleic acid (MA). These samples are both simpler and independently better characterized than the terpolymers studied earlier, providing a better system for developing and testing approaches

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to quantitation. The methods developed are also tested using one of the AA/AMPS copolymers studied earlier.

2. Experimental

Except where noted, negative ion electrospray mass spectra were obtained using a Quattro II (Micromass, Manchester, UK) triple quadrupole mass spectrometer equipped with a standard coaxial probe (capillary voltage −2500 V) and a Z-source. Nitrogen was used as the nebulizing (20 L/h) and drying gas (300 L/h). The source block and desolvation temperatures were 110 and 150 °C, respectively. Cone voltages (CVs) between −10 and −120 V were used, as specified. MS/MS experiments employed nominal collision energies of 2–80 eV, with roughly 4×10^{-3} Torr Ar collision gas in the second (RF only) quadrupole. Samples were loaded into a 250 μL syringe (SGE, Austin, TX) and infused directly into the probe via a fused silica capillary (50 μm i.d.) at 5 μL/min using a Harvard Apparatus (South Natik, MA) model 22 syringe pump.

Preliminary scans of the entire mass range (up to 4000 Da) were used to select the appropriate ranges for subsequent multi-channel acquisition (MCA) scans. MCA spectra acquired over the selected region (20–30 scans per spectrum) were background-subtracted using a solvent blank. Reported ion intensity ratios represent averages from triplicate spectra; cited uncertainties and error bars are derived from propagation-of-error calculations based on one standard deviation for these triplicate ratios.

A limited number of spectra were acquired with a Q-Star XL hybrid (Q-TOF) MS/MS system (Sciex, Toronto, ONT, Canada) equipped with an ionspray source. The instrument was operated in the MS mode (quadrupole analyzer operating with RF only), and other parameters were optimized for sensitivity, as follow: ionspray voltage, declustering potential, focusing potential, and declustering potential 2 were set at −4200, −200, −265, and −50 V, respectively; ion source and curtain gas flow rates were maintained at 1.36 and 1.13 L/min, respectively; and ion release delay and ion release width times were 123.90 and 59.14 ms. Infusion rates were again 5 μL/min. Spectra were acquired over the mass range of 50–3000 Da in the negative ion mode.

The SS/MA copolymers (nominally 1:1 or 3:1 SS:MA; 20,000 Da average molecular weight) were obtained from Aldrich (Milwaukee, WI) and used as received. The AA/AMPS copolymer was purified chromatographically as described previously [12]. All sample solutions were prepared in 1:1 (v/v) methanol/water (both water and methanol solvents were HPLC grade from Aldrich) containing 75 μM sodium hydroxide (Aldrich). Polymer samples were prepared at concentrations of 1.9 or 19.1 μg/mL; for the SS/MA copolymers, this constitutes roughly 0.1 and 1.0 μM, respectively. (The molecular weight and therefore the molar concentration of the AA/AMPS copolymer were unknown.) Samples used to generate calibration curves for monomer mixtures were prepared such that the total monomer concentration was maintained at either 10 or 40 μM while the concentrations of the individual monomers (Aldrich) were adjusted to obtain the desired molar ratio. Limits of detection (2σ LODs) were estimated by using sensitivities from monomer calibration curves (40 nM–2 μM) to estimate the concentration that gave a signal that exceeded that for a (copolymer-containing) blank by twice the standard deviation of the blank.

3. Results and discussion

Initial experiments with the SS/MA copolymers followed the protocol described previously [12]. The resulting low-CV (−20 V) spectrum for the 3:1 copolymer is shown in Fig. 1a; the spectrum of the 1:1 copolymer (not shown) differed slightly but reproducibly in the exact positions of the maxima. Polymodality like that evident in Fig. 1a was not observed in any of our earlier work wherein convolution of distributions of polymer mass, charge, isotopes, and monomer ratios resulted in *single*, unresolved spectral envelopes [12]. The polymodal structure could be attenuated but not removed by tuning the quadrupole or source optics, suggesting that it may reflect an unusual sample composition (e.g., resulting from blending of various polymer samples). However, examination of the same samples using a Q-Star hybrid Q-TOF instrument resulted in the expected single unresolved envelope (Fig. 1b and corresponding data for the 1:1 copolymer). The

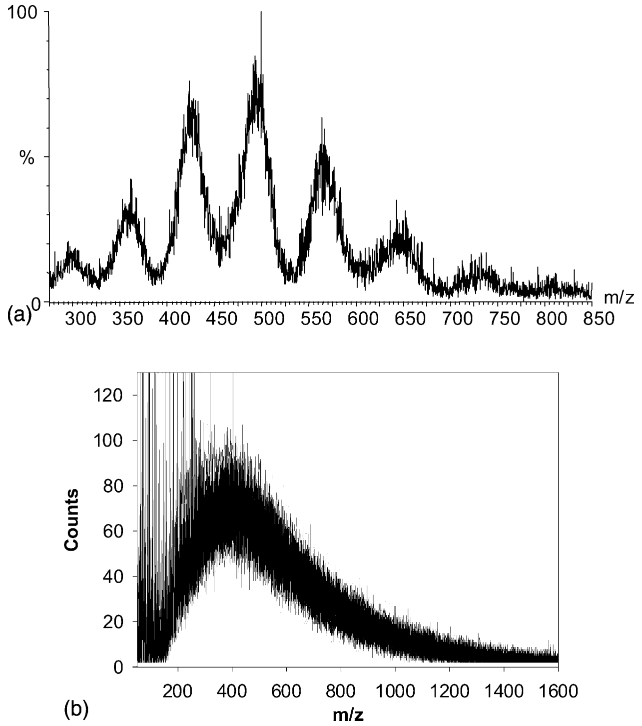


Fig. 1. ES mass spectra (high mass range) of a nominally 3:1 poly(styrene sulfonate-co-maleic acid) copolymer (19.1 mg/mL) obtained with (a) CV = −20 V on the Micromass instrument and (b) low energy conditions on the Applied Biosystems instrument (see text for specific voltages).

polymodality was also absent in earlier, preliminary studies of these samples using a Micromass "pepper pot" electrospray source [23]. We conclude that it is attributable to the performance of an RF-only lens that was installed when upgrading the Quattro from the "pepper pot" to the Z-source.

The complexity in the shape of the envelope did not complicate the use of in-source collision-induced dissociation (CID) for assessment of monomer ratios, along the lines reported in our previous study [12]. Fig. 2 compares portions of high (−100 V) and low (−20 V) CV spectra of the 1:1 copolymer; data for the 3:1 copolymer (not shown) were similar. As evident in the inset to Fig. 2a, very small amounts of monomer-related ions could be detected at *m/z* 115 and 183 (anions of MA and SS, respectively) even in the low CV spectrum—another contrast with earlier work. These small signals may be due either to residual monomers or to depolymerization at low CV. They disappeared below background at still lower CV, where the signal-to-noise ratio also became worse (probably due to poorer ion focusing). It can be concluded that the concentration of residual monomers must be less than the ~0.1 μM monomer LODs at these low CVs (Table 1). Consistent with this observation, comparison with monomer calibration curves acquired at CV −20 V (not shown) indicates that approximately 0.08 μM MA and 0.09 μM SS would be required to generate the monomer signals of Fig. 2a.

As observed with the terpolymer [12], adjusting the CV to −100 V sharply attenuated the polymer envelope while increasing the abundance of ions at *m/z* 115 and 183. By comparison with a calibration curve for SS monomers at CV −100 V (not shown), it can be estimated that roughly 2.4 μM SS monomer would have been required to generate the signal at *m/z* 183 in Fig. 2b—more than an order of magnitude higher than the SS monomer limit-of-detection in the presence of 1:1 copolymer at CV −10 V (~87 nM; Table 1). The corresponding SS concentrations for the 3:1 copolymer are 6.7 μM for data analogous to Fig. 2b, versus an LOD of 80 nM in the presence of the 3:1 copolymer; again, the monomer signals must derive from depolymerization, and residual monomer concentration in the polymer sample must be negligible.

Table 1
Limits of detection (LOD; nM) for the indicated monomer in electrospray mass spectra obtained at low cone voltages in the presence of 19.1 μg/mL copolymer (3:1 or 1:1 poly[styrene sulfonate (SS)-co-maleic acid (MA)]). Slightly higher LODs reflect the poorer signal-to-noise ratio that likely results from poorer ion focusing at CV −10 V

Monomer	CV (V)	LOD (nM) in the presence of	
		1:1 Copolymer	3:1 Copolymer
SS	−10	87 ± 21	80 ± 17
SS	−20	54 ± 15	35 ± 4
MA	−10	104 ± 29	118 ± 34
MA	−20	34 ± 8	44 ± 14

The case is slightly more complex for MA. As for acrylic acid in Reference [12], fragmentation of free MA monomers was so extensive that no analyte signal was detectable above background when sampling MA *monomers* at CV −100 V, regardless of concentration (see below). At this CV, the MA monomer apparently generates fragments below the effective low-mass cut-off of the Quattro Z-source (~*m/z* 52) and/or neutrals. Only energy dissipation by *polymer* fragmentation enabled generation of stable MA ions at *m/z* 115 at CV −100 V; all of the signal at *m/z* 115 in Fig. 2b is therefore attributable to monomers generated by depolymerization.

Having confirmed attribution of all or most of the signals at *m/z* 115 and 183 in Fig. 2b to depolymerization, it remained to assess the relation between the observed ion intensities and the sample monomer ratios.

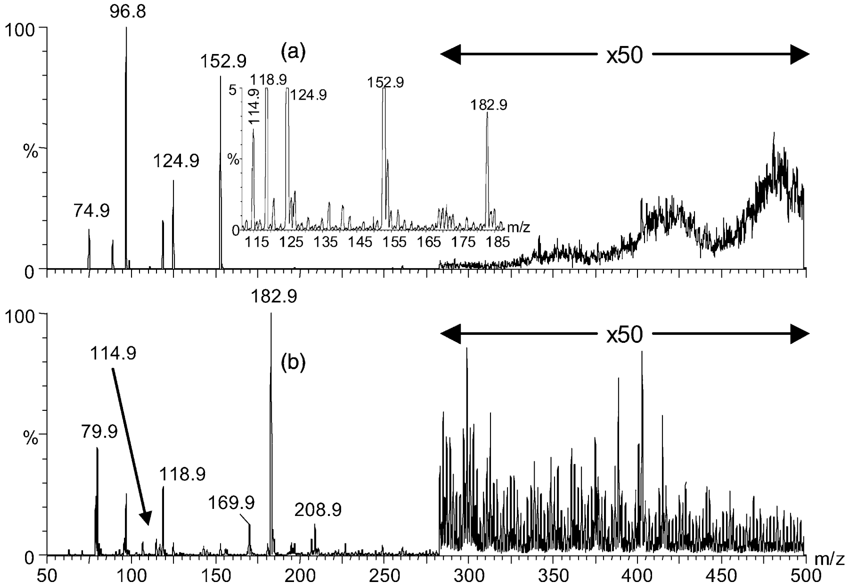


Fig. 2. ES mass spectra (low mass range) of a nominally 1:1 poly[styrene sulfonate-co-maleic acid] copolymer (19.1 μg/mL) obtained with the Micromass instrument at (a) CV = −20 V and (b) CV = −100 V.

Table 2
Experimental monomer ratio estimates derived from mass spectral data at the indicated cone voltages (CV) for (nominally) 1:1 and 3:1 poly[styrene sulfonate (SS)-co-maleic acid (MA)] copolymers (19.1 μg/mL)

CV (V)	Standard	Nominal monomer ratio (SS:MA)			
		1:1		3:1	
		r[a]	Σ[b]	r[a]	Σ[b]
−20	Monomer[c]	2.45 ± 0.97	2.81 ± 1.17	6.01 ± 2.75	6.96 ± 3.26
	Polymer[d]	1.19 ± 0.62	1.19 ± 0.62	2.52 ± 1.56	2.52 ± 1.56
−30	Monomer[c]	1.37 ± 0.32	2.09 ± 0.58	2.17 ± 0.30	4.86 ± 0.49
	Polymer[d]	1.81 ± 0.35	1.31 ± 0.24	1.66 ± 0.47	2.28 ± 0.64
−40	Monomer[c]	0.01 ± 0.29	0.92 ± 0.15	0.60 ± 0.30	3.08 ± 0.23
	Polymer[d]	0.79 ± 0.06	0.94 ± 0.07	3.82 ± 0.30	3.25 ± 0.29
−50	Polymer[d]	0.84 ± 0.06	0.92 ± 0.09	3.57 ± 0.29	3.27 ± 0.29
−60	Polymer[d]	0.64 ± 0.07	0.89 ± 0.11	4.71 ± 0.59	3.37 ± 0.46
−70	Polymer[d]	0.74 ± 0.08	1.01 ± 0.05	4.06 ± 0.48	2.98 ± 0.19
−80	Polymer[d]	0.70 ± 0.13	0.91 ± 0.13	4.29 ± 0.83	3.29 ± 0.46
−90	Polymer[d]	0.74 ± 0.10	0.93 ± 0.10	4.05 ± 0.52	3.22 ± 0.39
−100	Polymer[d]	0.66 ± 0.02	0.78 ± 0.03	4.57 ± 0.18	3.83 ± 0.19
−110	Polymer[d]	0.56 ± 0.03	0.73 ± 0.06	5.28 ± 0.43	4.07 ± 0.48
−120	Polymer[d]	0.69 ± 0.14	0.78 ± 0.11	4.32 ± 1.02	3.82 ± 0.71

[a] Estimates using ratios of intensities of intact monomer ions ($r \equiv i_{183}/i_{115}$).

[b] Estimates using ratios of sums of intensities for ions associated with each monomer: $\Sigma \equiv \frac{i_{183}+i_{119}+i_{80}}{i_{115}+i_{71}}$.

[c] Estimates using calibration curves from Fig. 6.

[d] Estimates using the 1:1 copolymer as a one-point calibrant for the 3:1 copolymer and vice versa.

The relative intensity of the SS monomer ions from the 1:1 SS:MA copolymer exceeds that of MA ions substantially; $r_{1:1\text{polymer}} \equiv (i_{m/z\,183}/i_{m/z\,115})_{1:1\text{ polymer}} = 18.50 \pm 0.64$. Greater sensitivity to SS is consistent with its relatively high acidity; $\mathrm{p}K_\mathrm{a} = 0.7$ for benzenesulfonic acid (which should resemble that for SS), versus 1.92 for MA [24]. The apparent bias toward the stronger acid was even greater for the 3:1 copolymer run under identical conditions; $r_{3:1\text{polymer}} = 84.64 \pm 1.83$. Thus, use of the 1:1 copolymer as a standard for assessing the monomer ratio in the 3:1 sample would have lead to overestimation of the ratio (84.64/18.50 = 4.57 ± 0.18; Table 2) at CV −100 V. Conversely, quantitation of the 1:1 sample as an "unknown" using the 3:1 sample as the standard would have resulted in underestimation of the 1:1 ratio (0.66 ± 0.02; Table 2). Depolymerization at CV −100 V is apparently sample-dependent.

Further study will be needed to determine the detailed reason for this dependence. It may result from variation in the efficiency of depolymerization with varying abundance of SS–SS, MA–MA, SS–MA, and MA–SS links, since these bonds likely differ in energy. Even if depolymerization is complete, the amount of energy dissipated in the process may be less if weaker bonds are more prevalent. If enough internal energy remains after depolymerization, polymer-derived monomers may subsequently fragment, thereby lowering the measured abundance of the more "delicate" monomer; given the absence of detectable ions when sampling MA monomers at CV −100 V, MA would thereby be underrepresented. The effect could be enhanced in the 3:1 copolymer due to the stronger acidity of SS (see above); molecules rich in SS may be more highly charged and therefore more effectively accelerated. The effect may also be enhanced by the higher conductance (304 ± 3 μS versus 289 ± 4 μS) and slightly higher emission current (31 ± 11 nA versus 26 ± 14 nA) associated with the 3:1 (versus the 1:1) copolymer solution; more aggressive in-source CID is generally associated with higher conductance [25]. The last effect is apparently small; lowering the copolymer concentration by an order of magnitude (to 1.9 μg/mL) had little or no effect on conductance or currents, which are evidently controlled by the added NaOH. Furthermore, the "ratio of ratios" ($r_{3:1}/r_{1:1} = 5.21 \pm 0.56$) obtained at low polymer concentration was not significantly different from that obtained at the higher concentration (4.57 ± 0.18). To better characterize contributions of varying energy deposition, and to seek conditions better suited to quantitation, the energy dependence of monomer and polymer fragmentation was studied.

3.1. Breakdown diagrams

"Breakdown diagrams" of fractional ion intensities for the SS and MA monomers (i.e., fraction of total analyte ion intensity attributable to a given ion) versus CV are shown in Fig. 3. The SS monomer (Fig. 3a) provides a "classical" example of a diagram for competing direct fragmentation and rearrangement [26] (183 → 80 or 183 → 119; Scheme 1). Assignments were confirmed by MS/MS characterization of the monomer ion at *m/z* 183 (data not shown), in which case the optimum intensity of the ion at *m/z* 119 occurred at a collision energy of about 25 V, versus CV approximately −60 V in Fig. 3a (reflecting the higher efficiency of energy deposition in the quadrupole collision cell, relative to in-source).

The MA monomer breakdown diagram (Fig. 3b) shows evidence for a 115 → 71 process (confirmed by MS/MS, and

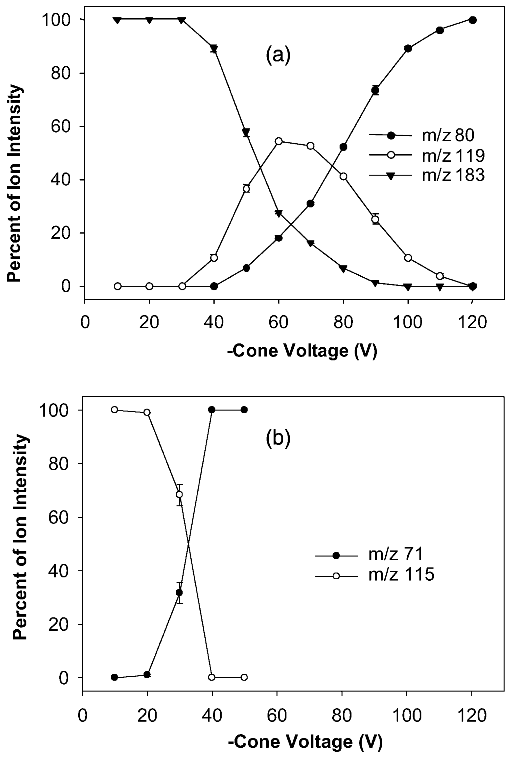


Fig. 3. Fractional fragment ion intensities (“breakdown diagrams”) for (a) styrene sulfonate and (b) maleic acid monomers. Each was obtained from a 5 μM solution of the corresponding monomer. Intensities are relative to the sum of intensities for the indicated analyte. The curve in (b) is discontinuous above CV −50 V because no analyte ions could be detected at higher energies.

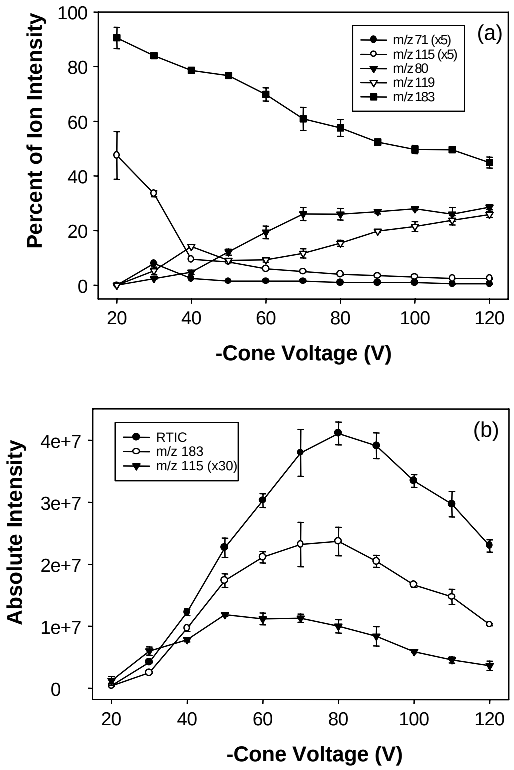
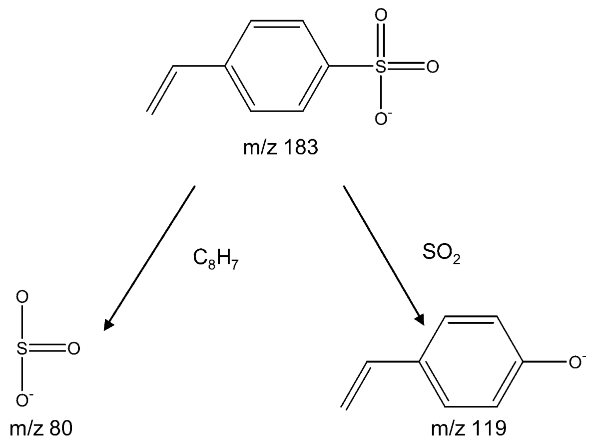


Fig. 4. (a) Fractional fragment ion intensities (“breakdown diagram”) for a nominally 3:1 poly[styrene sulfonate (SS)-co-maleic acid (MA)] copolymer (19.1 μg/mL). Intensities are relative to the sum of intensities for both the SS and MA monomers. The curve is discontinuous below CV −20 V because no monomer-related ions could be detected above the background. MA ions (*m*/*z* 115 and 71 represented by filled and unfilled circles, respectively) have been multiplied by a factor of five. (b) Cone voltage dependence of the absolute intensity of the base peak (*m*/*z* 183), the MA anion (*m*/*z* 115), and the reconstructed total ion current (RTIC).

consistent with simple loss of neutral CO_2 from the deprotonated maleic acid). At CV more negative than −50 V, the diagram must be abruptly terminated, as neither of these ions nor any others attributable to MA can be detected. Conceivably, the ion at *m*/*z* 71 may fragment further (e.g. by loss of CO_2 to form $C_2H_3^-$), generating fragments below the low-mass cut-off of the Z-source. Such behavior would be consistent with the electron ionization mass spectrum of acrylic acid [27], but no such fragments were detected in MS/MS spectra of precursors at *m*/*z* 71 or 115 from MA monomer (data not shown), even though the mass range of the second analyzer extends down to *m*/*z* 2.0. An alternative explanation for the disappearance of sample-related ions in Fig. 3b may be generation of solvent-related species capable of neutralizing the ion of *m*/*z* 71 (e.g., by protonating it to form acrylic acid, pK_a 4.25 [24]). Further evaluation of this possibility lies outside the scope of the current study.

The SS/MA copolymer breakdown diagrams differ from either monomer diagram in that fragment ions at *m*/*z* 71, 80, and 119 never dominate (data for the 3:1 copolymer are shown in Fig. 4a; similar trends were observed for the 1:1 copolymer). Due to energy dissipation by polymer fragmentation, the SS ion at *m*/*z* 183 remains the most abundant even at high CV. The greater acidity of the SS monomer is reflected in consistently greater abundance of SS-related ions; a multiplier of 5 has been applied to the MA ions to facilitate plotting on the same axes.

Plotted for comparison in Fig. 4b are the absolute intensities of the polymer-derived SS and MA monomer ions, and



Scheme 1.

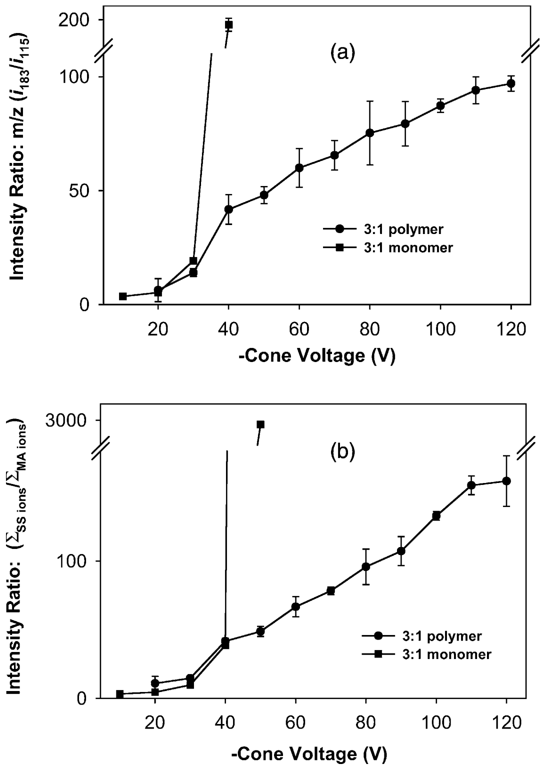


Fig. 5. (a) Measured monomer-ion intensity ratio and (b) total ion intensity ratio (including fragments) vs. CV obtained with a (■) 3:1 SS:MA monomer mixture and a (●) 3:1 poly[styrene sulfonate (SS)-co-maleic acid (MA)] copolymer.

the reconstructed total ion current (RTIC = the sum of the absolute intensities of the monitored ions) versus CV. (The MA absolute intensity was multiplied by a factor of 30 to facilitate plotting on the same axes.) Each curve is observed to pass through a maximum, reflecting increased polymer sampling and depolymerization as the CV increases initially, followed eventually by increased fragmentation. The MA ion intensity peaks well before that of the SS ion, reflecting the relative "delicacy" of MA monomers (due at least in part to their tendency to lose CO_2). Fig. 5

3.2. Quantitation using monomer and fragment ions

Detection of additional fragments (besides the monomer anions at *m/z* 115 and 183) suggests a way to increase the overall intensity and possibly improve quantitative performance by including the contributions of these other ions. Included in Table 2 are values of monomer ratios estimated as described above, but using total monomer ion ratios ($\Sigma \equiv \frac{i_{m/z183}+i_{m/z119}+i_{m/z80}}{i_{m/z115}+i_{m/z71}}$) instead of the simple monomer anion ratios ($r_{1:1}$ and $r_{3:1}$, as defined above). The precision of values derived from Σ generally increases with increasing −CV, reflecting the overall increase in fragment ion intensity at high CV. Moreover, use of Σ values between CVs of −40 and −90 V provides estimates of monomer ratios within experimental error of expected values when one copolymer is used as a standard for the other. The accuracy and precision of these estimates (column labeled "Σ" and rows labeled "polymer" in Table 2) are optimum at CV = −70 V, probably reflecting a compromise between the optimum intensity of the MA ions (at CV −50 V) and that of the SS ions (at CV −80 V).

reports the CV dependence of the intensity ratios $r_{3:1\text{polymer}}$ and $\Sigma_{3:1\text{polymer}}$ (as defined above). Included for comparison is corresponding data for a 3:1 SS:MA monomer mixture. The polymer data is reported only at $-\text{CV} \geq 20$ V; at lower energies, there is not sufficient depolymerization to provide measurable low-mass signals. Conversely, the *r* curve for the monomer mixture is interrupted for $-\text{CV} > 40$ V (where the peak at *m/z* 115 disappears; Fig. 3b), and the monomer Σ curve stops when $-\text{CV} > 50$ V (where no detectable ions were derived from MA monomers, as noted above). The close match of the initial portion of the curves in Fig. 5a and (especially) Fig. 5b suggests that at moderate cone voltages, the CID behavior of monomer mixtures and copolymers is similar. (A comparable match was observed for the 1:1 copolymer and a 1:1 monomer mixture; data not shown.) This in turn suggests the possibility of using monomer mixtures as calibrants for assessment of monomer ratios in the copolymers, in the absence of copolymer standards.

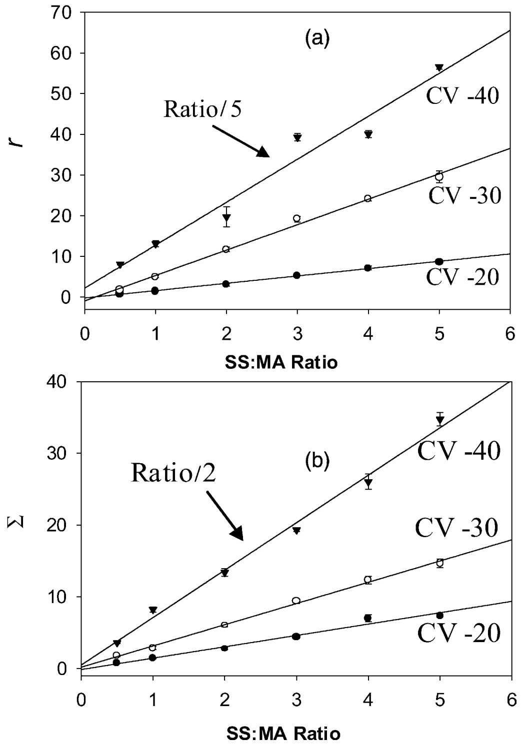


Fig. 6. Measured monomer ion intensity ratio at the indicated CV vs. the monomer concentration ratio for SS/MA monomer mixtures (total monomer concentration = 40 μM). (a) Simple intensity ratio $r \equiv i_{183}/i_{115}$. (b) Summed intensity ratio $\Sigma \equiv (i_{183} + i_{119} + i_{80})/(i_{115} + i_{71})$. The curves for CV of −40 V were divided by constants of (a) 5 and (b) 2.

Table 3
Slope, intercept, and R^2 values for calibration curves (40 μM total monomer concentration) shown in Fig. 6 (r) or (Σ), as appropriate

CV (V)	Calibration curve	Slope	Intercept	R^2
−20	r	1.8 ± 0.1	−0.2 ± 0.2	0.9962
	Σ	1.6 ± 0.1	−0.1 ± 0.4	0.9945
−30	r	6.2 ± 0.2	−0.9 ± 0.7	0.9953
	Σ	3.0 ± 0.1	0.2 ± 0.2	0.9971
−40	r	52.8 ± 5.1	11.0 ± 15.6	0.9635
	Σ	13.2 ± 0.5	1.0 ± 1.7	0.9930

Table 4
Quantitation of monomer ratios for nominally 1:1 and 3:1 poly[styrene sulfonate (SS)-co-maleic acid (MA)] samples using Σ ratio monomer calibration curves (Fig. 6b) obtained with either 10 or 40 μM total monomer concentration at CV = −40 V

Polymer concentration	10 μM calibration curve		40 μM calibration curve	
	1:1 SS:MA	3:1 SS:MA	1:1 SS:MA	3:1 SS:MA
1.9	1.04 ± 0.11	2.92 ± 0.20	1.07 ± 0.14	3.14 ± 0.24
19.1	0.94 ± 0.12	2.99 ± 0.20	0.92 ± 0.15	3.08 ± 0.23

Polymer concentration was either 1.9 or 19.1 μg/mL, as indicated.

3.3. Monomers or polymers as standards

Fig. 6 presents "calibration curves" of r (Fig. 6a) and Σ (Fig. 6b) versus monomer ratio at various CVs, each constructed using data from a series of solutions prepared with varying ratios of SS and MA monomers and a total monomer concentration of 40 μM. Note that the slopes of all of the curves are significantly greater than one (Table 3), consistent with the higher acidity of the SS monomer, cited above. The slopes correlate with CV, suggesting maximum sensitivity at highest energy. Curves for r are steeper than those for Σ, but the latter are generally more nearly linear (higher R^2, except at CV −20 V). Calibration curves obtained with lower total monomer concentration (10 μM, data not shown) did not differ significantly from those of Fig. 6 and Table 3, suggesting an absence of ionization suppression effects at these low concentrations.

The "Monomer Standard" data of Table 2 are obtained when the various 40 μM calibration curves are used to estimate monomer ratios from ion intensity ratios for the two polymer samples at the corresponding CV. The poor precision displayed for all values at CV −20 V results from the low intensity (S/N ~3) of monomer ions derived from the polymer at this CV (see Fig. 2a). Although the monomer calibration curves of Fig. 6 are well behaved (respectable R^2 values for both r and Σ calibration curves; Table 3), there is not sufficient polymer-derived monomer to afford an accurate measurement at CV −20 V.[1] At CV −30 V, precision is improved for all values, but the accuracy is poor. The observation of only a slight attenuation of the polymer envelope at −30 V indicates that the polymer may not be representatively sampled under these conditions (consistent with the low RTIC observed at this CV; Fig. 4b).

Increasing the CV to −40 V generates more depolymerization, affording enhanced sampling of the copolymer; the RTIC more than doubles when CV changes from −30 to −40 V. However, the accuracy and precision for the monomer-based calibration using r at this energy are poor, reflecting the low intensity at m/z 115 at this CV (Fig. 3b). By contrast, ratios based on Σ obtained at CV −40 V are all within experimental error of the nominal value. The Σ ratio compensates for the differing energetics of fragmentation of free monomer and monomer derived from polymer, thereby affording a much more accurate value, regardless of whether monomers or polymers are used as standards.

Significantly, the same results (within experimental error) are obtained regardless of whether the polymer samples contained 1.9 or 19 μg/mL (Table 4). Like the monomers, the polymers appear to be free of non-linear suppression effects at these low concentrations.

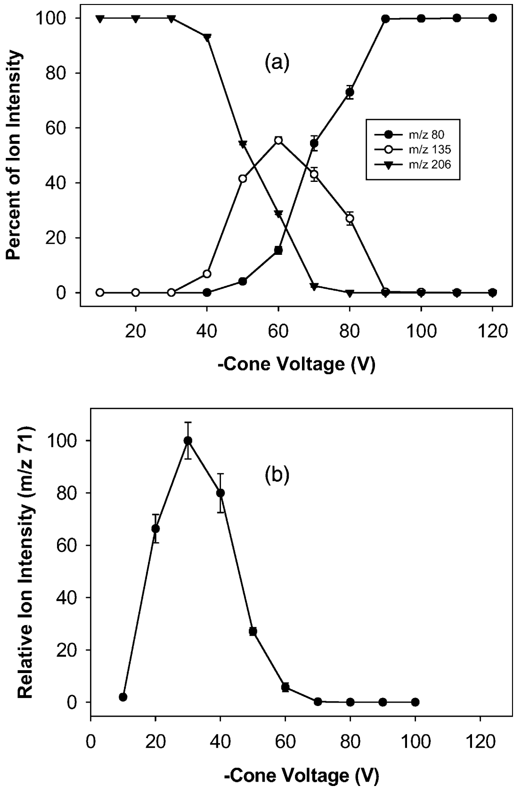


Fig. 7. (a) Fractional fragment ion intensities (breakdown diagram) vs. CV for AMPS. (b) Normalized ion abundance vs. CV for AA (m/z 71). Each curve was obtained from a 5 μM solution of the corresponding monomer. Intensities for (a) are relative to the sum of intensities for AMPS. The curve in (b) is relative to the highest absolute intensity observed for AA.

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[1] The apparent accuracy of the polymer-calibrated data at CV −20 V is clearly fortuitous in light of the low precision (relative standard deviation = 83% and 62% for the 1:1 and 3:1 sample, respectively). Agreement of r- and Σ-derived values results from the absence of monomer fragments at −20 CV.

3.4. Second application: poly(AMPS-co-AA)

The results above suggest a general approach to quantitating monomer ratios in copolymers, in which the monomer breakdown diagrams are used to determine the highest CV where all monomers exhibit considerable intensity; this CV is then chosen for analysis. A copolymer of AMPS and AA (Sample 7 in our previous study [12], wherein the AMPS:AA ratio was determined to be 1.80 ± 0.19, based on ^{13}C-NMR) provided a second test for the use of breakdown diagrams to optimize CID conditions for assessment of monomer ratios. Fig. 7 reports the relevant monomer breakdown diagrams. The curve for AMPS is remarkably similar to that for SS (Fig. 3a). For AA, the ion at *m*/*z* 71 was the only detectable ion, and it became undetectable at $-\mathrm{CV} > 60$ V. CV = −50 V was chosen for calibration because it produces significant attenuation of the polymer envelope and reasonable signal for the AA monomer. Based on a monomer calibration curve for Σ at this CV (considering ions at *m*/*z* 80, 135, and 206 for AMPS and *m*/*z* 71 for AA), and the corresponding ratio for the copolymer, the MS-derived monomer ratio was calculated to be 1.51 ± 0.31, within experimental error of the ^{13}C NMR result. While the precision is not high, it is clear that monomers can be used as standards for quantitating monomer ratios for both the SS:MA and the AMPS:AA copolymer systems.

4. Conclusions

These findings suggest that electrospray ionization mass spectrometry conditions can be found under which monomers can serve as standards for assessing the composition of copolymers in the absence of polymer standards. Once optimum conditions are identified, sample analysis takes only seconds, suggesting potential utility for routine monitoring of monomer ratios. All monomers in this study were acidic; extension to non-ionic polymers may be feasible, but certainly will not be trivial. Even application to other ionic polymer systems will require careful assessment of the fragmentation behavior of both the monomer and polymer as a function of CV.

Finally, it is noteworthy that in the course of these studies, differences between the fragmentation observed when sampling pure monomer versus monomer derived from depolymerization became clearly evident (compare, for example, Figs. 3 and 4). This suggests potential use of the monomer ion-to-fragment ion ratio as a measure of the concentration of residual monomers in a polymer sample. In light of the low LOD’s for monomers evident in Table 1, the utility of this alternative measure of contamination may be limited to diagnostics accompanying assessment of monomer ratios. Possible applications utilizing homopolymers are currently under investigation; results will be reported in a later publication.

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