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Interference from multiple cations in MALDI–MS spectra of copolymers

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Abstract

A matrix-assisted laser desorption/ionization Fourier transform mass spectral (MALDI–FTMS) investigation of copolymers of methyl methacrylate (MMA) and butyl methacrylate (BMA) is reported here. A key to the analysis of copolymers by mass spectrometry is the accurate assignment of a unique composition to each ion signal observed in a spectrum. In the present case, the spectra reveal numerous ions with nominal mass separations of 16 Da. This results from the presence of oligomers differing in composition by three extra MMA units and two lesser BMA units. Lithium–sodium and sodium–potassium adduct pairs produce ions that also differ by a nominal mass of 16 Da. Therefore, ambiguous assignments of compositions are possible, especially when the identities of the cations are unknown. When cesium ions are added to the sample, the adducts produced allow high resolution Fourier transform mass spectra to be used for assignment of unambiguous compositions to ions. Spectra with overlap from multiple cations were compared with the cesium adduct spectra using a mathematical analysis of relative ion abundances to determine the fraction of ionization that each cation contributed. When two cations were simultaneously added to a sample, the resulting fraction of ionization by one of the cations was related to the ratio of the two added salts. This relationship is well described by an empirical equation that allows the prediction of the ionization fraction for a given ratio and vice versa.

Keywords: MALDI; Copolymers; Cation; Interference

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1. Introduction

Polymers are ubiquitous in modern society, with innumerable applications. Bulk physical properties of polymers are determined by their molecular properties; particularly, the chemical composition of the monomer units, the stereotacticity of side groups, the molecular weight distribution of the polymer mixture, and the endgroup identity. Therefore, the accurate determination of molecular properties is an essential part of polymer study and design. The monomer identities of an unknown polymer can sometimes be determined by using nuclear magnetic resonance or the fragmentation patterns of secondary ion mass spectrometry, while size exclusion chromatography is frequently used to determine the overall distribution of molecular weight in the bulk polymer. However, matrix-assisted laser desorption/ionization (MALDI) coupled with time-of-flight (TOF) or Fourier transform mass spectrometer (FTMS) mass analyzers allow for more direct analysis of both size and the composition of polymers. In MALDI experiments, neutral polymers are charged by complexation with protons or metal cations which generally complex in a 1:1 stoichiometric ratio.

Mass-to-charge ratios can be determined with reasonable mass accuracy (100–200 ppm) and adequate resolving power (10,000 FWHM) using a MALDI–TOF instrument, and with better mass accuracy (<30 ppm) and resolving power (200,000 FWHM or greater) using an FTMS instrument. Typically, both are capable of analyzing polymers that contain only one type of repeating unit. The mass of each ion is composed of oligomer repeating units plus the terminating groups and the attached cation; for homopolymers, this mass can be used to infer a unique composition even when relatively low resolution measurements are made because the possible compositions are separated by several mass units. The abundances of the ions reflect, in theory,

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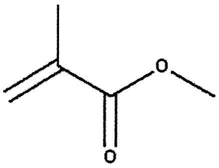
the relative abundances of oligomers with different masses, and therefore, the molecular weight distribution. In this paper, the problem of accurately assigning MALDI ion composition and cation identities by high resolution FTMS is addressed.

Analysis of copolymers, polymers containing more than one type of repeating unit, is significantly more complicated than for homopolymers. As an example, consider the case of oligomers containing 10 monomer units: there is only one such 10-mer if only a single monomer is considered. There are 11 possible 10-mers using two starting monomer species (disregarding permutations of the placement of the monomers), and 66 possibilities if three monomer species are considered. Some combinations of the monomers will occur more frequently than others, so not all of the possibilities will be observed. However, it is clear that MALDI spectra of copolymers will contain many more distinct ions than will the spectra of homopolymers. It is important to be able to determine the composition and relative abundance of each oligomer in a copolymer because these characteristics play an important role in determining the bulk physical properties of the polymer. For instance, the block lengths of block copolymers largely determine the core size of their resultant micelles [1]. The distribution of block lengths can only be deduced if the composition and abundance of the oligomers are ascertained correctly. In order to study any property of a polymer that is dependent on the composition of its oligomers, mass spectral ion assignments must be made unambiguously.

Several researchers have discussed the ambiguity of compositional assignment when two or more distinct oligomers have masses that closely agree with the observed mass-to-charge ratio of an ion in a mass spectrum [2]. This problem is exacerbated by less accurate mass measurements or low resolution data. Multiple assignments can be particularly problematic at high mass because more assignments are possible, each oligomer has a wider isotopic envelope, and the resolving power of mass spectrometers is often reduced at high mass. Some of these complications can be mitigated by obtaining higher resolution FTMS data to determine which oligomers are actually present at a given mass. If TOF is used as the mass analyzer or if FTMS resolution is still insufficient, assignments of compositions can frequently be elucidated using data analysis techniques. In his general review of copolymer mass spectrometry, Montaudo cites several reported methods to determine correct assignments [2]. One simple method uses “pruning” to eliminate certain compositions that are considered unlikely [3,4]. Another method uses a statistical approach that analyzes the spectrum as a whole to give expected contributions of different oligomers at the same mass [5,6].

The ambiguities in mass assignments discussed above are a consequence of actual differences in composition of polymers having very similar molecular weights. Multiple assignments may also arise from a change in composition and a simultaneous replacement of one cation for another [7]. This is particularly problematic for lithium, sodium, and potassium. Replacing lithium with sodium increases the mass by 15.9738 Da. Replacing sodium with potassium increases the mass by 15.9739 Da. If only integer mass is considered, any two monomers whose greatest common factor is also a factor of 16 will have oligomers that differ by 16 mass units because the linear combination of two relatively prime integers spans the set of integers. Frequently, the 16 Da shift is improbable: for instance, in a copolymer of vinyl acetate (VA) and ethyleneglycol methacrylate (EGMA), a 16 Da shift would occur if 20 VA monomers were removed and 26 EGMA monomers were added to a particular oligomer. However, with other pairs of monomers, a change in composition resulting in a 16 Da shift may be quite likely. In the present study, we analyzed a random methyl methacrylate (I: MMA) and butyl methacrylate (II: BMA) copolymer, for which a 16 Da shift occurs when three MMA units are added and two BMA units are removed.



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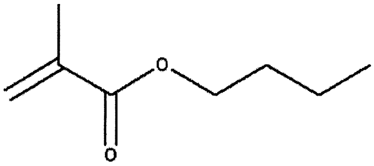


II

Table 1 represents some combinations of monomers that show 16 Da differences between two oligomers. Because the masses of the overlapping ions are so close, one can easily assign the wrong cation and the wrong composition to an observed ion, yet calculate excellent agreement between the observed masses and the expected masses of their incorrect assignments. Additionally, isotope patterns could not be used to assign compositions because sodium and cesium each have a single naturally abundant isotope, while ^{7}Li and

Table 1
Ion compositions and corresponding mass differences

Composition with attached potassium	Composition with attached sodium	Mass difference between oligomers	Resolving power needed at 3000 m/z
$(ST)_m(MMA)_n$	$(ST)_{m+4}(MMA)_{n-4}$	0.067	45,000
$(MMA)_m(BA)_n$	$(MMA)_{m+4}(BA)_{n-3}$	0.015	194,000
$(MMA)_m(BMA)_n$	$(MMA)_{m+3}(BMA)_{n-2}$	0.015	194,000
$(MMA)_m(MAAC)_n$	$(MMA)_{m-5}(MAAC)_{n+6}$	0.015	194,000

ST: styrene; MMA: methyl methacrylate; BA: butyl acrylate; BMA: butyl methacrylate; MAAC: methacrylic acid.

^{39}K constitute about 93% of the natural abundance for their respective elements. It is then necessary to make cation assignment based on information outside the spectrum (e.g., the assumption that a particular indigenous cation dominates the ionization, or that a high enough concentration of an added cation will eliminate contributions from all others).

In addition to the possibility of making an incorrect assumption about the cation attachment in a copolymer, it is also possible that more than one cation may contribute to the ionization of the sample. This would result in two oligomers of different compositions contributing to the ion abundance observed at a single mass. If this occurs, then, an assignment based on the attachment of a single cation will not reflect the true composition of the polymer. The extent to which the data is skewed is a function of the fraction of attachment of the interfering cation and the amount of compositional change resulting in a 16 Da shift. This effect could distort determinations of average block lengths in block copolymers, average mole fractions of monomers in random copolymers, or kinetic observations made from the spectra.

One strategy to circumvent this difficulty is to add an excess of one cation so that ionization from other cations is suppressed [8]. Another strategy is to add cations whose oligomer-attached masses do not overlap those from other cations. In the present research, two cations (silver and cesium) were added giving cationized oligomer masses close to those of theoretical masses of lithium-, sodium-, and potassium-adducts of other oligomers. However, the difference between the masses is such that a resolving power of 10,000 or 20,000 is sufficient to distinguish the two different ions with m/z value of 3000. This resolution is easily obtained using an FTMS instrument, but lies out of the range of most TOF instruments. Because the oligomers cationized with silver or cesium can be unambiguously identified by the accuracy of their mass determination, the abundances of these ions accurately reflect the distribution of different compositions within the polymer. Accordingly, for composition analysis, other cationized species can be ignored. This distribution can be used as a standard to which lithiated, sodiated, or potassiated spectra can be compared in order to evaluate the contributions of interfering cations that distort the observed intensity pattern. In the experiments described here, cesium gave higher abundance spectra than silver, so three cesiated spectra were averaged to use as the standard.

2. Materials and methods

2.1. Instrumentation

All MALDI experiments were performed using a HiRes Ultima-external source (IonSpec Co., Lake Forest, CA, USA) FTMS equipped with a 9.4 T actively shielded magnet. For the desorption–ionization process, a pulsed Nd-YAG laser (New Wave, Fremont, CA) operating at 355 nm was used. The laser power is 2 mJ per pulse at 100% attenuation. All experiments were performed with a laser attenuation setting of 25% resulting in an average energy of 500 μJ per shot. Spectra were acquired in positive ion mode at pressures of 4.0×10^{-13} to 7.0×10^{-13} bar. Each spectrum is the sum of five transients, and each transient measured ions accumulated from six laser pulses [9]. A gated trapping scheme was used in which the trapping interval was incremented by 100 μs with each laser shot to include the entire mass range of the polymer [10].

2.2. Chemicals and reagents

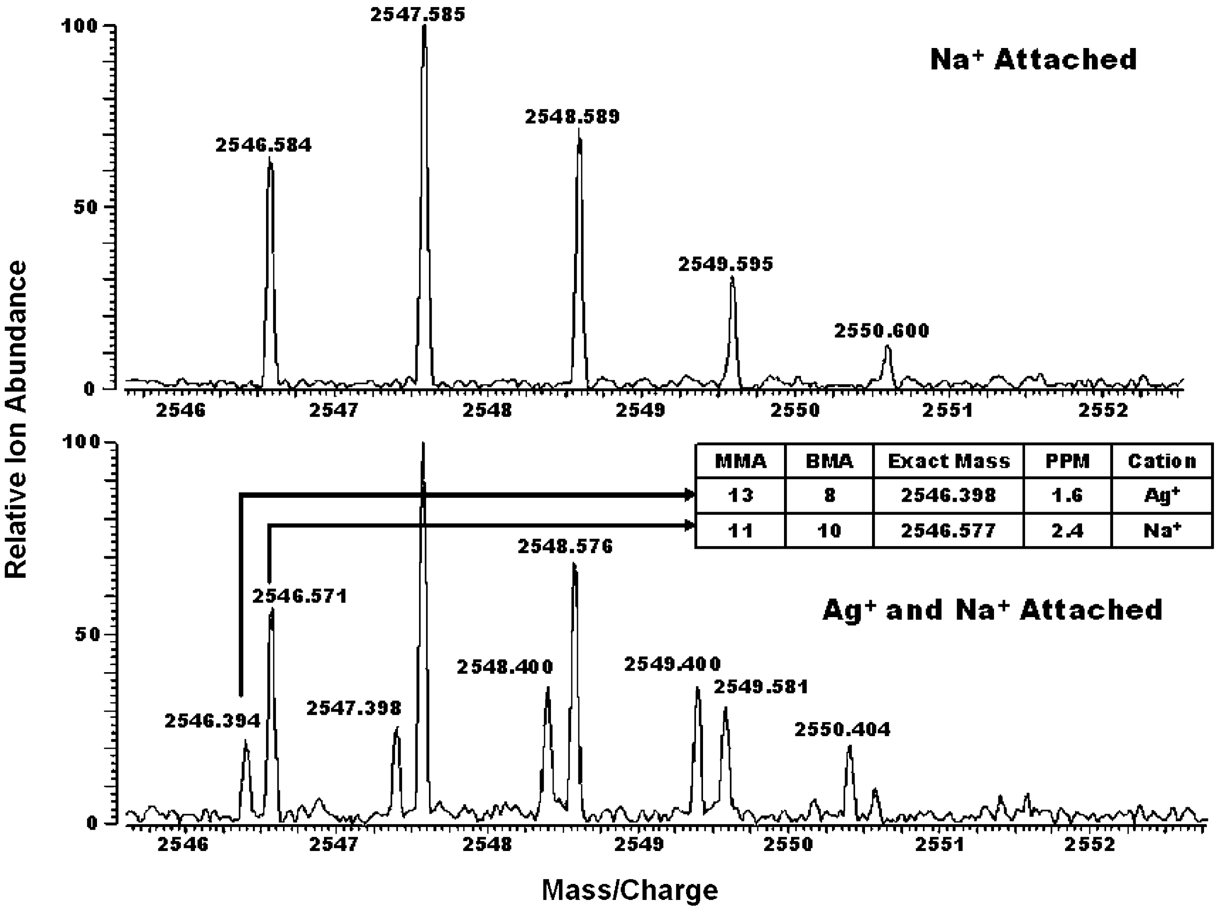
Methyl methacrylate/butyl methylacrylate (MMA–BMA) random copolymer was obtained from DuPont Marshall Research and Development Laboratory (Philadelphia, PA) where it had been synthesized via group transfer polymerization [11]. Lithium chloride, sodium chloride, and potassium chloride were purchased from Sigma (St. Louis, MO, USA) and cesium chloride from IBI Shelton scientific, Inc. (Shelton, CT, USA). Silver nitrate was obtained from Aldrich (Milwaukee, WI, USA). 2,5-Dihydroxybenzoic acid (DHB) was obtained from Fluka (Milwaukee, WI, USA). All reagents were used without further purification, with the exception of DHB, which was purified for the spectra in Figs 2–5A. In Fig. 5A, one set of samples used DHB that was purified by adding concentrated hydrochloric acid (10 ml) to a saturated aqueous solution of DHB (10 ml) and extracting the solution three times with diethyl ether (20 ml aliquots). In Figs. 2–4 and a second set of samples in Fig. 5A, DHB was purified by sublimation under an aspirator vacuum at 200 °C. Unpurified DHB was used to obtain the data for Fig. 1, the third set of samples in Fig. 5A, and the samples used to produce Fig. 5B and C.

2.3. Sample preparation

The LiCl, NaCl, and KC1 salts were prepared in 50% aqueous methanol solution to make concentrations of 100, 40, 20, 10, and 5 mM solution. The MMA–BMA copolymer was dissolved in methanol to result in a concentration of 6 mg/ml or approximately 2 mM. DHB matrix was dissolved to a concentration of 1.0 M in methanol. For samples containing two cations, the alkali chloride salts (100 mM) were mixed in (v/v) ratios of 10:1, 5:1, 2:1, 1:1, 1:2, 1:5, and 1:10. The MALDI sample was prepared using the “dried droplet” method by mixing 2 μl of the copolymer solution with 2 μl of the salt solution and 2 μl of matrix in a micro-centrifuge tube. Two microliters of the resulting mixture was deposited on the MALDI target plate and allowed to air dry before insertion into the FTMS.

2.4. Data analysis

Relative abundances of the ions from three interference-free spectra were averaged and used to prepare a table of



MMA	BMA	Exact Mass	PPM	Cation
13	8	2546.398	1.6	Ag^+
11	10	2546.577	2.4	Na^+

Fig. 1. Region of two MALDI-9.4T-FTMS spectra obtained in broadband mode showing the isotopic envelope of a sodium-attached MMA–BMA oligomer (top and bottom) and the closely spaced but clearly resolved ions from a silver-attached oligomer of different composition (bottom).

theoretical masses and abundances for lithium, sodium, and potassium adducts (not shown). This table was compared with experimental data using Eq. (1).

$$\mathrm{Int}_i = N(A(\mathrm{Int}_{\mathrm{A},i}) + (1 - A)(\mathrm{Int}_{\mathrm{B},i})) \qquad (1)$$

The parameter N is a normalization factor to account for the difference in total ion abundance between one spectrum and another, the parameter A is the fraction of ions with cation A attached, while the remainder of ions are assumed to be cation B adducts. The three intensities at mass "i" are the experimental (Int_i), the theoretical with cation A attachment ($\mathrm{Int}_{\mathrm{A},i}$), and the theoretical with cation B attachment ($\mathrm{Int}_{\mathrm{B},i}$). Least-squares optimization of parameters in all fits was performed using the nonlinear regression feature of Mathematica 5.0 ©. Eq. (1) considers the possibility of attachment from only two different cations. When lithium, sodium, and potassium were included simultaneously, the best fit parameters were calculated with a high degree of uncertainty. Therefore, each fit included only the cations that were deemed most likely to be present in the sample. For Figs. 3, 4A, 5B and C, no lithium was added so each spectrum was assumed to have only sodium and potassium. For Figs. 5A and 4B, lithium was added, so lithium and sodium were assumed to constitute most of the ionization. However, a small amount of error may be included in the lithium/sodium calculations since they neglect the possibility of a contribution by potassium.

3. Results

In order to form the table described in Section 2.4, spectra had to be obtained with high signal-to-noise ratios and with ion abundances that were free from the chemical interference produced by undesired cations. As discussed above, both silver and cesium ion adducts have masses that can be resolved from adducts of other cations using FTMS in the mass range of this polymer which produces ions with mass-to-charge ratios between 1300 and 4200. The top spectrum in Fig. 1 shows the isotopic envelope of a sodium-attached oligomer containing 11 methyl methacrylate monomer units and 10 butyl methacrylate monomer units. Silver nitrate has been added to the sample from which the bottom spectrum was obtained; silver-attached ions containing 13 MMA monomers and 8 BMA monomers are clearly resolved from the more abundant ions from the sodium adducts which have not been displaced by silver. The resolution needed to separate desired adducts from sodium adducts was obtained for spectra of ions with either cesium or silver attached. However, addition of cesium chloride gave excellent spectra with high signal-to-noise ratios and almost complete displacement of native sodium and potassium cations, whereas, silver nitrate and silver trifluoroacetate gave less intense spectra with low signal-to-noise ratios and only partial displacement of native cations. Additionally, silver attachment would complicate the analysis described in Section 2.4 because of its two abundant isotopes. Therefore, spectra of cesium-attached ions rather than silver-attached were chosen as standards to represent the actual abundances of the different polymer compositions. Three spectra of cesiated oligomers were analyzed by assigning compositions to all ions from a list of the masses for all possible oligomers with hydrogen endgroups and cesium attachment. Hydrogen endgroups were assumed because group transfer polymerization is known to produce that result [11]. A total of 751 ions, including their isotopes, were observed in all three spectra and gave good agreement with expected masses (less than 35 ppm error over the entire mass range). The relative abundances of these ions were averaged, reducing shot-to-shot variation to give a reference spectrum. A representative spectrum for the copolymer with cesium-attached ions is shown in Fig. 2. Because of the spectral complexity, visually similar spectra result in the absence of cesium.

The abundances of these ions were used to calculate the relative contributions of sodium, potassium, and lithium in other experimental spectra. To accomplish this, the averaged reference spectrum was translated along the mass axis into theoretical spectra for lithium, sodium, and potassium attachment by subtracting the mass of cesium and adding the mass of the appropriate second cation to the mass associated with each signal observed in the spectrum. Experimental spectra were compared to these theoretical spectra, and a least-squares analysis by Eq. (1) was used to determine the fraction of ions that were formed as potassium, lithium, and sodium adducts. For instance, if an experimental spectrum has no cationization except from potassium, then the abundances will correlate well with those calculated for potassium adducts, but not with those corresponding to lithium or sodium attachment. On the other hand, if half the ionization is caused by potassium and half by sodium, then, the abundances will be correlated, but less strongly, with the theoretical abundances for both sodium attachment and potassium attachment, but will correlate most strongly with a theoretical spectrum where half the abundance of each sodium-attached ion is added to half the abundance of each potassium-attached ion at the same mass. This is demonstrated in Fig. 3 which shows the region from *m*/*z* 2700–2800 of the experimental (3A, 3B, and 3C) and theoretical spectra (3D, 3E, and 3F) for complete sodium ionization, complete potassium ionization, and a mixture that was determined by Eq. (1) to contain 43% sodium adducts and 57% potassium adducts. For these analyses, and those described subsequently, it is assumed that the polymer's affinities for different cations are constant over the 2900 Da mass range of the MMA–BMA copolymer. Spickermann et al. found limited cation mass bias effects for narrow polydispersity (1.03–1.05) poly(methylmethacrylate) with differences in calculated average molecular weight ranging from 1 to 13%, depending on the mass range chosen [12]. When chloride counterions were used (as in the present study) [13], it showed only "marginal variation" for a low polydispersity (1.07) sample of MMA over the mass range 1000–4000 Da. The present study had about a 7% variation between the extremes. Thus, the assumption does not introduce significant error for narrow polydispersity samples (1.04) such as those analyzed here.

Two sets of experiments were conducted to observe the effect of multiple cations on spectra of MMA–BMA copolymers. In the first experiment, two salts were added to the

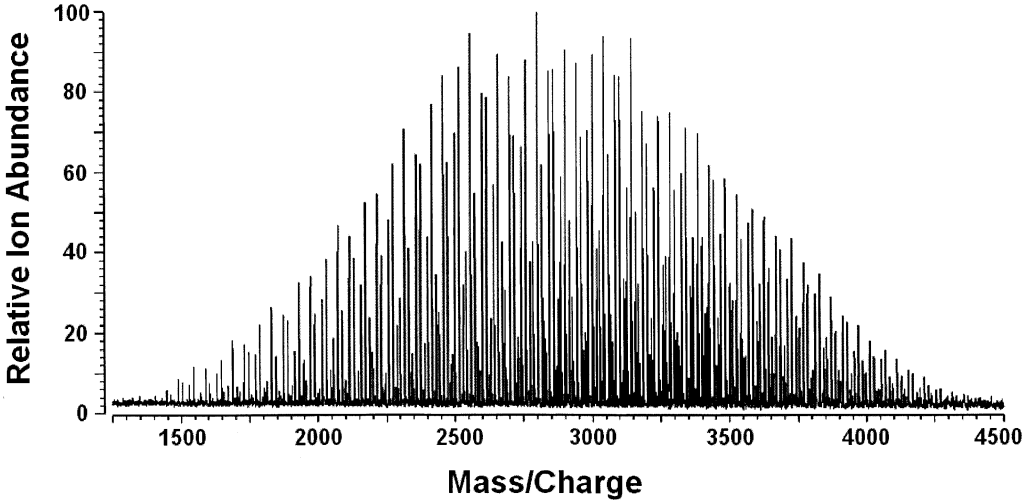


Fig. 2. A broadband MALDI-9.4T-FTMS spectrum of MMA–BMA copolymer with predominant cesium attachment to ions.

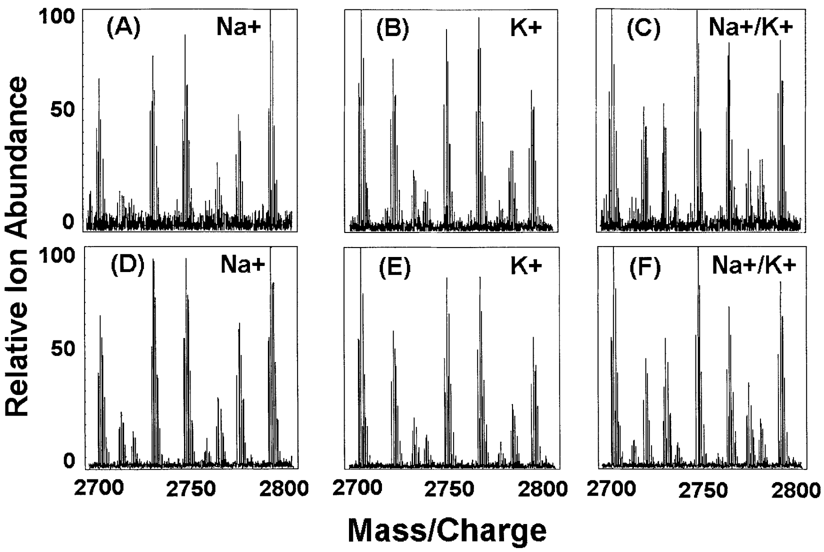


Fig. 3. Regions of MALDI-9.4T-FTMS spectra between 2700 and 2800 for samples showing only sodium attachment (A), only potassium attachment (B), and a mixture of both (C). The masses of cesium-attached ions in the reference spectrum were translated to give theoretical intensities for sodium (D) and potassium (E) attachment. Eq. (1) calculated that the mixture containing 57% potassium attachment and 43% sodium attachment giving rise to the theoretical spectrum shown in (F).

same sample with a 1:50 ratio of analyte to total salt concentration. In one sample, sodium and potassium were added to the analyte mixture; in the other sample, sodium and lithium were added. Different ratios of the two salts were used, ranging in concentration from 10:1 to 1:10. A 1:500 molar ratio of analyte to sublimed DHB was used in this experiment. Fig. 4 plots the fraction of ionization by potassium (4A) and lithium (4B) versus the molar ratio of these salts to sodium chloride. The abscissas are shown in log scale to evenly space the points. The observed trends in ionization are described by the following empirical equation.

$$\text{Fraction of ionization} = \frac{A(\text{salt ratio})^B}{1 + A(\text{salt ratio})^B} \qquad (2)$$

The salt ratio is determined by the sample preparation; the fraction of ionization is calculated using Eq. (1). A and B are parameters of the fit. For a particular cation, the parameter A is related to the affinity of the copolymer for that cation relative to the copolymer's affinity for the other cation added (which was sodium in our experiments). Three to five spectra were obtained at each salt ratio, and these data points were then fit using Eq. (2). This model accounts for approximately 80% of the variance from the mean ($R^2 \approx 0.80$) for the data. However, when the fraction of ionization from several points at the same salt ratio is averaged, the model accounts for greater than 95% of the variance from the mean. This indicates that Eq. (2) describes the observed relationship quite precisely as most of the

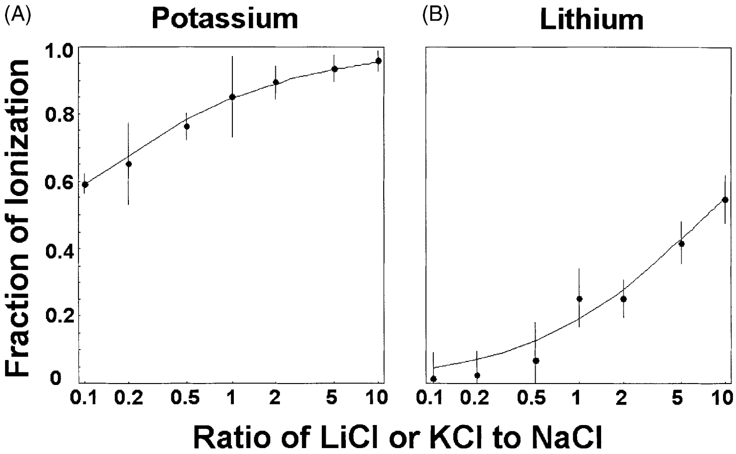


Fig. 4. Ionization by a particular cation vs. the molar ratio of that cation to sodium. Points and error bars indicate the mean and standard deviation for three to five replicates per ratio. Curves show best fit parameters using Eq. (2). For potassium sodium mixes (A), $A = 5.3 \pm 0.7$; $B = 0.58 \pm 0.08$; the individual points show an R^2 value of 0.82, and the means of the points at each ratio show an R^2 value of 0.99. For lithium sodium mixes (B), $A = 0.24 \pm 0.03$; $B = 0.71 \pm 0.08$; the individual points show an R^2 value of 0.80 for this fit, and the means of the points at each ratio show an R^2 value of 0.96.

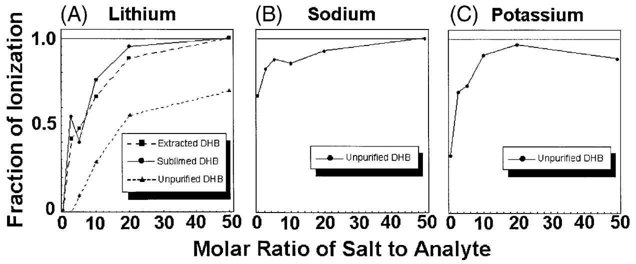


Fig. 5. The fraction of ionization by a particular cation is plotted against the salt-to-analyte ratio added to each sample. The effects of matrix purification are shown for additions of lithium chloride (A). For addition of lithium (A) and potassium (B), the interfering ionization was assumed to be completely due to sodium (see Section 2.4). For the addition of sodium, the interfering ionization was assumed to be due to potassium.

variance from the model can be accounted for by shot-to-shot variation.

In the second set of experiments, only one salt of lithium, sodium, or potassium was added to a sample of MMA–BMA copolymer. The analyte-to-salt ratio was increased from 1:2.5 to 1:50 for all the three salts, while the analyte-to-matrix ratio was held at 1:500. The relative ionization contribution of the added cation was then calculated by Eq. (1) for each of the salt concentrations used. For lithium salts, three to five spectra were acquired at each salt ratio; the averages of these points are plotted in Fig. 5A. However, for sodium and potassium salts (Figs. 5B and C), only two data points were taken at each ratio. Samples with added lithium and potassium had large amounts of competing ionization from adventitious sodium. In order to reduce the amount of competing sodium, DHB was purified using sublimation and extraction as described in Section 2.2. Spectra using these purified DHB matrix solutions were compared to those using non-purified DHB matrix to see the extent of sodium ionization in spectra of samples containing lithium chloride. This is shown in Fig. 5A. The Eq. (2) best fit parameters determined for lithium sodium mixes (Fig. 4B) were used to calculate the apparent concentration of sodium in the samples prepared with non-purified DHB, extracted DHB, and sublimed DHB. The salt ratio for each sample preparation is equal to the concentration of added lithium divided by a background sodium concentration which is assumed to be constant. The sodium concentration for unpurified DHB was calculated to be 2.4 ± 0.4 mM ($R^2 = 0.78$). The extracted DHB had a concentration of 0.30 ± 0.08 mM ($R^2 = 0.83$), and the sublimed DHB had a sodium concentration of 0.25 ± 0.09 mM ($R^2 = 0.63$). This indicates that purifying the matrix removed between 85 and 90% of the background sodium concentration in the sample. The residual sodium could very likely have been present in the polymer. The DHB used to obtain the spectra in Fig. 5B and C was not purified. The samples with sodium added were analyzed (including the 2.4 mM background concentration of sodium in samples with unpurified DHB) to determine the concentration of potassium native to the system. This concentration was calculated to be 0.019 ± 0.005 mM ($R^2 = 0.65$). The fractions of sodium ionization for sodium doped samples are plotted in Fig. 5B. The fraction of potassium ionization for potassium doped samples is plotted in Fig. 5C.

4. Discussion

The analysis described in Section 2.4 hinges on the primary assumption that the relative abundances of different oligomers of the MMA–BMA copolymer are reflected by the abundances of ions formed and detected in the MALDI process, regardless of other experimental parameters. If this assumption is correct, then any cation that attaches to the copolymer will reflect the same compositional distribution, although the signals in the mass spectrum will be shifted to different masses. This would allow the detection of multiple cations and their relative ionization contributions quantified by Eq. (1). However, if experimental parameters such as laser fluence affect the ion distribution, or if different cations strongly bias different mass regions, then, the assumption would be false. This could introduce systematic or random error into the results of Eq. (1).

Nevertheless, results derived from Eq. (1) predictably correlate with experimentally controlled salt ratios (Fig. 4). Random variation in the fraction of cation attachment can be overcome by increasing replicate trials of the experiments. Systematic variation in cation attachment, in particular due to laser fluence can be measured with controlled experiments and may be the subject of a future publication.

Fig. 4 demonstrates that the ratio of cation attachment is well-described (R^2 greater than 0.95) by the empirical Eq. (2). The results from Eq. (2) can be used to calculate the salt ratio that gives rise to an observed ionization ratio. This method is employed to determine the amount of sodium present in the samples prepared with unpurified and purified matrix solutions demonstrating that purification removed a substantial amount of background sodium. The same method predicts that a 19 μM background concentration of potassium cation could account for the potassium ionization seen when only sodium chloride is added to a copolymer sample (Fig. 5B). The sodium and potassium concentrations indigenous to samples are determined with relatively high error. However, even with this error, the predicted ionization ratios agree well with the observed ionizations in Fig. 4B and C.

These competition experiments demonstrate that a MMA–BMA copolymer has a greater ionization affinity for potassium chloride than for sodium chloride, which in turn is greater than for lithium chloride. These observations agree with the conclusions of Hoberg et al. that poly-MMA exhibits the following order of affinities for alkali metal chlorides: Cs > Rb > K > Na > Li that was attributed to differences in the lattice energy of the salts [13]. It should be noted that our experiments were carried out using DHB matrix, while Hoberg et al. used dithranol. The choice of matrix is significant as it has been shown to strongly affect the relative cation affinity of poly(ethylene glycol)s (PEG) [14]. If these observations extend from PEG systems to acrylate systems, then, our results may be less applicable to samples prepared with matrices other than DHB.

Use of a mathematical description of the ionization may allow for a quantitative description of the MMA–BMA affinity for different salts. This can be used, for instance, to calculate the theoretical salt ratio needed to attain 95% ionization by a particular salt by extrapolating the equations shown in Fig. 4A and B. If sodium and lithium are mixed, then, an 8:1 excess of sodium is sufficient to give 95% sodium ionization, whereas, a 500:1 excess of lithium is required to give 95% lithium adducts. For potassium/sodium samples, a 9:1 excess of potassium and a 2500:1 excess of sodium are required to show 95% ionization by the desired cation. This assumes that the best fit parameters describe the ionization curve at all salt ratios because we extrapolate beyond the ratios used in this experiment.

The data show that even high salt-to-analyte ratios may not be effective in completely removing interferences from competing cations that can be indigenous to the analyte or matrix solution or can be introduced during sample preparation. Even if only sodium chloride is added, interference from micromolar concentrations of potassium persisted until a 50:1 salt-to-analyte ratio was used. In order to increase the ionization yield by a particular cation, two basic strategies can be adopted: the required ratio of desired salt to competing salt can be lowered by using a cation for which the polymer has a high affinity; or the actual salt ratio can be increased by adding excess of the desired salt or removing competing salts by purifying the matrix or other components of the sample.

However, the required salt ratio may be strongly dependent on several factors including choice of matrix, laser fluence, and monomer composition. It is impossible to tell solely from the observed MMA–BMA spectra whether multiple cations (from among lithium, sodium, and potassium) are present and, if so, what fraction they contribute to the total ionization. Although measures can be taken to favor attachment by a particular cation, the efficacy of these measures may be difficult to determine and may also be system dependent. Therefore, within the mass limits where suitable resolution can be attained, MALDI–FTMS with cesium cation can generate the most reliable spectra of the copolymers listed in Table 1, since FTMS has the resolving power to separate cesium-attached ions from sodium or potassium adducts that could otherwise compromise the analysis. TOF analysis of the copolymers in Table 1, and others with similar 16 Da mass spacing should be done cautiously with the knowledge that it may be difficult to remove these interferences even if measures are taken to reduce them.

5. Conclusions

FTMS analysis of MMA–BMA copolymers with added cesium chloride provides a convenient method for eliminating possible interference from the presence of multiple cations such as the sodium/potassium adduct pair. Qualitatively, cation affinities for the polymer studied fall in the order Cs > K > Na > Li. Eq. (1) allows for a novel analysis that determines the fraction of ionization from two different cations in a system where almost all the signals from one cation attachment overlap with that of the second. The empirical relationship (Eq. (2)) between salt ratio and ionization ratio was useful in interpreting the data presented in this work. It may also allow quantitative comparisons of the ionization efficiencies for different systems. For instance, one could investigate how different matrices affected the A and B parameters of Eq. (2), or how their values changed when using polymers of higher molecular weights, or different monomer compositions. If a systematic study was performed, this quantitative method would be preferable to a qualitative ordering of cation efficiencies.

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