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Enhanced Mixture Analysis of Poly(ethylene glycol) Using High-Field Asymmetric Waveform Ion Mobility Spectrometry Combined with Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

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Abstract

The combination of high-field asymmetric waveform ion mobility spectrometry (FAIMS) with Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) makes possible lower detection limits, increased sensitivity, and increased dynamic range in the analysis of poly(ethylene glycol) (PEG) samples of low molecular weight. The signal gain obtained using FAIMS depends on ion identity, with a range between 1.8× and 14× obtained for various molecular ions of PEG 600. A 1.7-fold reduction in noise is obtained using FAIMS due to the elimination of chemical noise. The improved detection performance is predominantly due to a reduction in adverse Coulomb effects as a result of ions being selectively introduced into the mass spectrometer. The high ion transmission obtained using FAIMS combined with the high sensitivity of FTICR-MS detection make possible separation of multiple gas-phase conformers of PEG molecular cations that have low abundance (less than 0.2% relative abundance) and that have not been detected previously. Mixed dications of PEG that have the same nominal mass but differ by the number polymer subunits ($m/\Delta m$ up to 25 000) can be separately introduced into the mass spectrometer using FAIMS. Interactions of the carrier gas with the metal ions that are attached to the PEG molecules appear to be the most significant factor in these FAIMS separations.

Many mass spectrometers, including ion trap, Fourier transform ion cyclotron resonance (FTICR-MS), orbitrap, and some hybrid instruments, store ions prior to or during mass analysis. Ion storage instruments are ideally suited for pulsed ionization methods, such as matrix-assisted laser desorption/ionization, because a complete mass spectrum can be obtained from a single ionization event, such as a single laser shot. These instruments are often capable of very high sensitivity and resolution, with over 8 000 000 resolution obtainable using FTICR-MS.^{1,2} With FTICR-MS, the high resolution is made possible because the time-dependent ion signal can be measured for very long times (up to several minutes³), which makes it possible to precisely determine the ion cyclotron frequencies. The signal is damped or decreases primarily due to a loss of coherence (dephasing) of the ion packet. This can occur via collisions with background molecules, magnetic or electric field inhomogeneities, and Coulomb interactions between ions. The latter phenomenon can produce other adverse effects, such as the coalescence of ion signal.^{4,5} The Coulomb repulsion between like-charged ions also limits the number of ions that can be stored in trapping instruments, which, in turn, limits the dynamic range of a measurement. Limited dynamic range is a key problem in the analysis of complex mixtures and has been described as “probably the greatest challenge for proteome analysis”.⁶

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Several methods to reduce the effects of the space charge problem in trapping instruments have been implemented. For example, automated gain control, which actively varies the duration of external accumulation⁷⁻⁹ or the duration of an ionization event,¹⁰ has been used to extend the dynamic range and sensitivity of ion trap and FTICR mass spectrometers. Other methods that have been used to increase the dynamic range in FTICR-MS include selective accumulation, where ions of interest are accumulated and separated based on their m/z prior to introduction into the ion cell by using a quadrupole or other mass-selective filter.¹¹⁻¹⁴ Similarly, the detection of low-abundance ions in a complex mixture can be enhanced by selectively accumulating these ions directly in the FTICR-MS cell.¹⁵

Another way to increase the dynamic range for trapping instruments is to combine mass analysis with chromatographic or electrophoretic separations that can be done either off-line or on-line using a variety of interfaces. For example, liquid chromatography (LC) when combined with FTICR-MS can extend the effective dynamic range of an analysis by 2 orders of magnitude.⁶ However, chromatographic or electrophoretic separations can often significantly increase the time required for analysis.

Ion mobility spectrometry (IMS) with a drift tube apparatus can be used to rapidly separate components in complex mixtures. Clemmer and co-workers have taken advantage of this method, in combination with nano-LC, to separate tryptic peptides from the *Drosophila* proteome. In these experiments, the time required for the LC separations was ~1 h, whereas the IMS separations take place in <10 ms. Ions with different charge states, but with similar m/z values, were readily resolved by IMS.¹⁶ Because these drift tube measurements separate ions predominantly on the basis of their collisional cross sections normalized to their charge state, there is a significant correlation between the size of the ion and its mass for a given charge state.

An alternate ion mobility method, pioneered by Guevremont and co-workers, is high-field asymmetric waveform ion mobility spectrometry (FAIMS).¹⁷⁻²⁰ This method has several interesting features compared to conventional IMS done in a drift tube, including the following: high ion transmission for high sensitivity,¹⁹ ease with which it can be adapted to virtually any type of mass spectrometer with minimal or no modifications to the instrument, and the ability to separate ions based on a combination of both the collisional cross section²⁰ and the chemical properties^{21,22} of the ion.

Briefly, FAIMS separations are based on the differential mobility of an ion at high versus low electric field.^{18,23} Ions drift between two electrodes on which an oscillating electric field is applied. This applied waveform consists of a high field of one polarity for a short time and a lower field of the opposite polarity for a longer time. The asymmetric waveform alternately attracts ions toward one and then the other electrode. If the mobility of an ion depends on the magnitude of the electric field, then there will be a net displacement of the ion toward one electrode. This net displacement can be compensated for by applying a dc offset potential, referred to as the compensation voltage (CV), to one electrode. The CV value corresponding to maximum transmission through the FAIMS apparatus depends on the identity of the ion. A CV scan is a series of mass spectra obtained as a function of CV values. FAIMS has been used to separate different ion conformers as well as different components present in mixtures, including small organic molecules,²⁴⁻²⁶ peptides,²⁷⁻³⁰ and proteins.^{20, 31-33}

Robinson and Williams recently combined FAIMS with FTICR-MS to selectively introduce different gas-phase conformers of ubiquitin ions into the mass spectrometer.³¹ In combination with H/D exchange in the ion cell, it was demonstrated that these methods resolved different populations in the conformer space; that is, these two methods are largely orthogonal. This

multidimensional separation made it possible to identify the presence of many more conformers than either method alone.³¹

Here, we demonstrate other advantages of combining FAIMS with FTICR-MS for the analysis of poly(ethylene glycol) (PEG), including improvements in sensitivity and dynamic range. In addition, we show that FAIMS can resolve disodiated versus diprotonated polymers of the same nominal mass as well as identify gas-phase conformers of PEG ions not previously detected.

EXPERIMENTAL SECTION

FAIMS Experimental Parameters

The Ionalytics Selectra (Ionalytics, Ottawa, Canada) was used for all FAIMS experiments. The FAIMS electrodes are cylindrical with inner and outer diameters of 16 and 20 mm, respectively. The electrodes have a hemispherical end through which ions pass into the entrance capillary of the mass spectrometer (Figure 1). The electric field from these curved electrode surfaces provides an ion focusing effect for enhanced sensitivity.¹⁹ The FAIMS electrode assembly slides onto the Bruker Apollo source (Bruker Daltonics, Billerica, MA) without modifying the source of the mass spectrometer (Figure 1). All FAIMS experiments were performed with the standard Ionalytics waveform consisting of a sine wave combined with the second harmonic 90° out of phase. This asymmetric waveform has a maximum amplitude, or dispersion voltage, of -4600 V and a frequency of 750 kHz. The nitrogen carrier gas was filtered by a molecular sieve and was maintained at a flow rate of 2.0 L/min.

Ion Generation

Ions were generated by nanoelectrospray ionization from pulled glass capillaries with tip openings of ~2 μm. The tip is positioned ~5 mm from the opening in the curtain plate on the FAIMS device, and 1000 V is applied to the curtain plate. About 2100 V is applied to a platinum wire placed inside the electrospray tip. All solutions contained 10⁻⁴ M PEG 600 (Aldrich Chemical, Milwaukee, WI) in water or 50/49/1 vol % water/methanol/acetic acid. Solutions with Li⁺, Na⁺, or Cs⁺ cations were formed by spiking the aqueous PEG 600 solution with 10⁻¹ M stock solutions of lithium hydroxide (Aldrich Chemical), sodium hydroxide, cesium chloride, or both (Fisher Scientific, Fair Lawn, NJ). Cation concentrations were ≤ 5 × 10⁻⁴ M. All chemicals were used as provided without further purification or processing.

PEG Characterization

PEG 600 is a synthetic linear polymer with a number average molecular weight (M_N) of 600 ± 30, as stated by the supplier. The polydispersity (PD) was reported to be 1.04 ± 0.03.³⁴⁻³⁷ Woldegiorgis et al. reported a weight average molecular weight (M_W) value of ~620.³⁴ To characterize PEG distributions, M_N , M_W , and PD were calculated using eqs 1-3, respectively.³⁸

$$M_N = \sum \frac{M_i N_i}{N_i} \quad (1)$$

$$M_W = \sum \frac{(M_i)^2 N_i}{M_i N_i} \quad (2)$$

$$PD = M_W / M_N \quad (3)$$

In these equations, M_i is the mass of the observed ion with i polymer subunits, corrected to the neutral mass with no charges, and N_i is the measured intensity of the ion. The M_N , M_W , and

PD distribution parameters were used to quantify ion abundance distribution shifts observed with and without the FAIMS device.

The mole fraction of PEG *n*-mers in solution can be calculated using the previously defined distribution parameters and the Gaussian molecular weight distribution function,

$$X(M_i) = \frac{1}{\sigma \sqrt{2\pi}} e^{-(M_i - M_N)^2 / 2\sigma^2} \quad (4)$$

where, $X(M_i)$ is the mole fraction of the PEG molecule with mass M_i .³⁹ The width of the Gaussian distribution, σ , is determined from eq 5.^{34, 40}

$$\sigma = \sqrt{(M_w M_N - M_N^2)} \quad (5)$$

The concentration in solution of specific PEG *n*-mers is calculated using these equations with M_N and M_w equal to 600 and 620, respectively. The calculated concentrations of various PEG *n*-mers were used to estimate sensitivity with and without FAIMS from the slope of a fitted line in S/N versus concentration plots.

FTICR-MS Parameters

All mass spectra were acquired on the Berkeley-Bruker 9.4-T Fourier transform ion cyclotron resonance mass spectrometer (Bruker Daltonics). Some details of this instrument have been previously published.⁴¹ Ions were accumulated in a hexapole ion trap for 0.4 s prior to injection into the ion cell of the mass spectrometer. Twelve ion injections were performed for each mass spectrum. Nitrogen gas was pulsed into the ion cell for 2 ms between ion injections. A data set size of 512K points was acquired, and the data set was Fourier transformed directly for all mass spectra. No scan averaging was performed. Instrumental parameters were reproduced as consistently as possible between different experiments because the observed PEG distributions have been reported to be sensitive to instrumental conditions.^{35,38,42}

Ions were trapped in an extended pseudo-open cylindrical (EPOC) cell.³¹ The EPOC cell is similar to other open cylindrical cells^{43,44} except that the region of the cell where ions are excited and subsequently detected is 150 mm, 2.4 times the length of the cylindrical trapping plates. Additional noncylindrical trapping plates have also been incorporated into the cell to allow ions to be trapped with essentially the same conditions as the standard Bruker Infinity cell. The source side trapping plate voltages were 3 V, and the trapping plate voltages on the side farthest from the source were 4 V. The EPOC cell provides significant improvements in both ion storage time and ion capacity over the Bruker Infinity cell.

CV Scans

Compensation voltage scans are automatically acquired using a custom XMASS Tk/Tcl script (Bruker Daltonics). The FAIMS device is set to a fixed compensation voltage, and a mass spectrum is acquired and saved. The process is then repeated at a different compensation voltage. A pumping delay between mass spectra was included to maintain the pressure inside the ultrahigh-vacuum chamber at $\sim 3 \times 10^{-9}$ Torr. Compensation voltages are increased linearly between scans with a step size of -0.1 V in a typical range from 0 to -20 V. All data processing was performed using R statistical analysis software (R Foundation for Statistical Computing, Vienna, Austria) version 2.0.1.

RESULTS AND DISCUSSION

Molecular Weight Distributions

An ESI mass spectrum of PEG 600 (10^{-4} M aqueous solution with 5×10^{-4} M NaOH) results in a distribution of predominantly singly sodiated molecular ions with a maximum abundance corresponding to the PEG 14-mer. The M_N and PD determined from these data (Figure 2d) are 622 and 1.02, respectively. The M_N value reported by the manufacturer is 600 ± 30 . An M_W of 620 and a PD value of 1.04 ± 0.03 have been measured for this sample.³⁴⁻³⁶ Polymer molecular weight distributions determined by mass spectrometry can potentially deviate from the “true” values due to mass bias, instrument conditions,^{35,42} effects of cation size,⁴⁵ and electrospray ionization solvents.⁴⁶ The results shown here, however, are in excellent agreement with previously reported values.

With FAIMS, a mass spectrum at any single CV value does not provide an accurate measure of the polymer distribution because not all ions are transmitted equally through the FAIMS device at a single CV value. This effect is illustrated in Figure 3 which shows two mass spectra at CV values of -7.4 and -5.9 V (Figure 3a and b, respectively). A more representative molecular weight distribution can be reconstructed from the CV data set by plotting the maximum signal obtained for each polymer cation obtained in the CV scan. These data are shown in Figure 2a. The 10-mer is the most abundant ion in this distribution, but the distribution is centered around the 13-mer. The M_N and PD of this distribution are 586 and 1.04, respectively. The M_N value derived from the FAIMS data is lower than that obtained without FAIMS, and the distribution is slightly wider. However, these values are also within the range of previously reported values.

For a direct comparison, the maximum ion signal for each polymer cation obtained from a set of 10 mass spectra without FAIMS is shown in Figure 2b. The distribution obtained from these latter data is nearly the same as the signal-averaged data without FAIMS (Figure 2d). Note that all the plots in Figures 2-4 have the same relative abundance scale so that quantitative comparisons between absolute ion intensities can be made. By comparing the reconstructed MW distribution with FAIMS (Figure 2a) to the MW distribution without FAIMS (Figure 2b), two important conclusions can be drawn. First, the abundance of any given ion that is transmitted through the FAIMS device is enhanced compared to that without FAIMS. Second, this signal gain depends on the identity of the ion and this causes the observed changes in the distribution of molecular ion abundances.

Signal and Dynamic Range Gain

The maximum signal for all polymer ions is increased when introduced through the FAIMS device compared to that without FAIMS. The greatest signal gain is a factor of 14 observed for the 7-mer, the least gain is 1.8 for the 16-mer. On average, there is a 4.3-fold increase in signal for the PEG cations when using FAIMS. This effect is illustrated for the 22-mer in Figure 4. A CV scan has a maximum intensity for this ion at -6.3 V (Figure 4c). A partial mass spectrum obtained at this CV value is shown in Figure 4b. By comparison, a partial mass spectrum without FAIMS is shown in Figure 4a. For this ion, FAIMS results in a 3.7-fold improvement in signal and a 6.3 total improvement in S/N. The higher level of noise apparent in the spectrum without FAIMS (Figure 4a) compared to that without FAIMS (Figure 4b) is due to fewer background ions entering the cell with FAIMS. The noise level with FAIMS is the same as that without FAIMS when no ions are introduced into the mass spectrometer. This demonstrates that FAIMS can eliminate chemical noise that is the limiting background when FAIMS is not used for this polymer analysis. Thus, FAIMS can improve S/N both by increasing absolute signal and by reducing chemical noise, a key advantage for complex samples. In

general, the S/N gain is most significant for lower abundance species. This S/N gain increases the number of n -mers that can be detected from 17 without FAIMS to 20 with FAIMS.

The signal gain could be due to ion focusing in the FAIMS device that makes it possible to introduce a greater number of ions into the mass spectrometer,^{19,47} or it could be due to improved performance of the mass spectrometer because ions are being selectively introduced into the mass spectrometer thereby reducing effects of charge–charge repulsion from other m/z species that are also in the cell. To distinguish between these two possibilities, the CV data were replotted by summing the ion intensity for each n -mer in spectra where the S/N was greater than 1.2 for that n -mer. On average, ~ 10 mass spectra were summed for each ion. These data are shown in Figure 2c. By comparison to results obtained by summing 10 spectra without FAIMS (Figure 2d), it is apparent that the absolute number of ions that are introduced into the mass spectrometer using FAIMS over the width of the CV peak is approximately the same as without FAIMS, although some ions are clearly enhanced using FAIMS. This enhancement is most notable for the low-abundance ions. Particularly apparent are both the PEG 7- and 10-mer, which are significantly enhanced with FAIMS. The optimum CV value at which these ions are transmitted is significantly different from optimum CV values for other ions (and different from each other). Thus, these ions are more efficiently separated from other ions with FAIMS. This results in an increased number of these ions being trapped in the cell relative to other ions generated by ESI. The selective accumulation of these ions results in a greater observed ion abundance relative to other ions that are marginally transmitted through the FAIMS device at these CV values. These results indicate that the signal gain obtained using FAIMS under these conditions is primarily the result of improved detection due to more efficient filling of the hexapole ion trap or ion cell with the ion of interest. This makes it possible to observe low-abundance ions that would otherwise be difficult to detect without FAIMS (see, for example, Figure 4). Thus, the dynamic range of a trapping instrument, such as a quadrupole ion trap or a FTICR mass spectrometer, can be extended by using FAIMS.

The concentration of each PEG n -mer in solution can be calculated using a Gaussian distribution function. For the 10^{-4} M PEG 600 solutions used here, the concentrations of the 13-mer and the 5-mer are 1.6×10^{-5} and 6.8×10^{-8} M, respectively. From these calculated concentrations, the sensitivity can be estimated from the slope of a linear fit to plots of n -mer S/N versus the calculated solution concentration for that n -mer (data not shown). The sensitivity obtained using FAIMS is ~ 2.4 times higher than without FAIMS using identical experimental conditions.

Gas-Phase Conformer Separation

In addition to the S/N gain possible with FAIMS, it is also possible to separate different conformers of the same ion. This effect is illustrated in Figure 5a, which shows a three-dimensional plot of m/z , CV, and ion intensity for the molecular cations of the 14-mer. A CV scan for m/z 657.37 (a “slice” from the three-dimensional plot) is shown in Figure 5b. Four conformers are observed, but one of these conformers is dominant with 98.9% relative abundance. Conformers I, III, and IV have 0.5, 0.4, and 0.2% relative abundance, respectively. It is important to note that these are conformers of the singly charged species; multiply charged clusters that have the same m/z ratio can be readily distinguished based on the spacing between the isotopes and no such clusters were observed in these experiments. It is possible that clusters survive the FAIMS process that takes place at atmospheric pressure and subsequently dissociate in the FTICR-MS instrument. However, this seems unlikely under the conditions of these experiments where non-specific clusters can be readily observed.^{41,48,49}

Similar results are obtained for many other n -mers. These data are summarized in Figure 6, which shows the maximum of peak intensity for these conformers as a function of CV value and n -mer size. Multiple conformers are observed for the 10–16-mers. The CV values of the

most abundant conformer become more negative with increasing polymer size, peaking at the 10-mer. These values subsequently become more positive with increasing polymer size. Bowers and co-workers found only a single conformer for each of the singly sodiated PEG 600 cations formed by laser desorption.^{50,51} The measured cross sections increased linearly with n -mer size.⁵² Detection of these additional, low-abundance conformers may be due to the excellent S/N obtained with FAIMS, the different ionization methods, or these conformers may have nearly identical collisional cross sections but can still be resolved using FAIMS. Although the physical cross section of an ion clearly must play a role in FAIMS separations,^{53,54} other factors, such as interactions between the ion and the carrier gas, also appear to play a role.^{21, 22} Previous studies have shown that gas-phase conformers of ubiquitin that have nearly identical collisional cross sections can be resolved based on their different extents of H/D exchange.³¹ De la Mora and co-workers found that collisional cross sections of much larger molecular weight PEG polymers correspond to one of several gas-phase conformations depending on the charge of the PEG ion, a result attributed to Coulombic repulsion in these multiply charged ions.⁵⁵ Our results are the first measurements of multiple gas-phase PEG conformations within a single charge state and where intramolecular Coulomb repulsion is absent.

Effect of Cation Size

In a separate experiment, CV scans were obtained for PEG 600 solutions to which Na⁺, Li⁺, and Cs⁺ were added, making possible simultaneous acquisition of these data for each of these three cationized species. From these data, the CV values corresponding to the maximum transmission of these ions as a function of polymer size are obtained (Figure 7a). These data are also plotted versus ion cross section (Figure 7b) using data reported by Bowers and co-workers for these ions,^{50-52,56,57} clearly showing that ion cross section and CV values are not directly correlated for this range of PEG n -mers. The CV values for the sodiated and cesiated species are increasingly negative with polymer size, reach a maximum at 10- and 13-mers, respectively, and then become more positive with further n -mer size. For the lithiated species, the CV values become more negative with increasing polymer size up to the 9-mer, but then these values are essentially constant for larger n -mers.

The metal ions interact predominantly with the oxygen atoms in the PEG molecules. The number of oxygen atoms in PEG that can optimally coordinate with the metal ions depends on both the metal ion and the polymer size. Bowers and co-workers have modeled cation-PEG interactions for 9-, 13-, and 17-mers and found that the optimum number of oxygen atoms that coordinate with the metal ion are 7, 8, and 11 for Li⁺, Na⁺, and Cs⁺, respectively.⁵⁶ However, these coordination numbers are only obtained for n -mers of sufficient size that suitable molecular geometries can be obtained. For example, sodium is coordinated to 7 of the 10 oxygen atoms for the 9-mer, but it is fully coordinated to 8 oxygen atoms for the 13-mer.⁵⁶

The polymer size necessary to fully coordinate the metal ion nicely correlates with the observed trends in CV values versus polymer size for these cationized species (Figure 7). These data peak at 9, 10, and 13 for Li⁺, Na⁺, and Cs⁺, respectively. These values appear to be very close to the number of subunits necessary to fully coordinate the metal ion. This result indicates that the trend in CV versus polymer size is likely due to effects of interactions of the carrier gas with the metal ions in the FAIMS device. When the metal ion is not fully coordinated, there should be significant interaction between the carrier gas and the metal ion. However, as the number of incorporated polymer subunits increases, the interaction between the metal ion and carrier gas becomes less significant due to the PEG polymer surrounding the metal ion, effectively shielding the metal ion from interactions with the carrier gas. For large n -mers, the CV values for Li⁺, Na⁺, and Cs⁺ are essentially indistinguishable, indicating that the metal ion interactions with the carrier gas are no longer a factor in the FAIMS separations. Minor

conformers are most likely due to ion structures that differ in how the polymer molecules interact with the metal ion. Interestingly, two of these minor conformer families for Na^+ appear to shield the ion less effectively, but one of the minor conformer families appears to solvate the ion more efficiently than the major conformer (Figure 6).

Doubly cationized species are also produced by electrospray ionization from these solutions. A plot of optimum CV values for transmission of each n -mer observed for dicesiated, disodiated, and the mixed dication is given in Figure 8. The size of the symbols in this figure indicates the relative abundance of each ion. These dication species show a similar trend of CV values versus polymer size as was observed for the singly cationized species, but the maximums in these curves are shifted to larger n -mer size. For disodiated, dicesiated, and the mixed dication, the maximums occur at 19, 22, and 21, respectively. The number of oxygen atoms in PEG that would be required to solvate two independent cations should be approximately twice that for a single ion, or 16 and 22, respectively. Again, the maximums in the CV values roughly correspond to the size of ion necessary to fully solvate the cations and thereby reduce the interactions of these ions with the carrier gas.

Resolving Power of FAIMS with FTICR-MS

An important advantage of FAIMS is the ability to separate ions that have the exact same mass.^{24,27} Even ions of very similar mass and composition can be separated. Figure 9 shows a three-dimensional plot of CV, ion intensity, and m/z (only a very limited range shown) resulting from a PEG water/methanol solution to which acetic acid and both Na^+ and Cs^+ cations were added. Doubly charged ions corresponding to the 20-mer with two protons, the 17-mer with one proton and one cesium ion, and the 19-mer with two sodium ions are observed at m/z ratios of 450.275, 450.184, and 450.243, respectively. These ions can be readily resolved using FTICR-MS (Figure 9a) based on m/z , but these ions can also be readily separated by FAIMS (Figure 9a,c). CV curves for each of these ions are shown in Figure 9c, clearly showing baseline separations for these ions. Figure 9b shows partial mass spectra measured at the CV value corresponding to maximum transmission for each of these three ions, demonstrating that interfering ions that are very similar in mass can be readily prevented from entering the FTICR-MS cell.

Similar results were obtained for PEG 1500. Figure 10a is a three-dimensional plot of CV, ion intensity, and m/z (limited mass range shown) corresponding to doubly protonated PEG 34-mer (m/z 758.458) and doubly sodiated PEG 33-mer (m/z 758.427). These ions differ in mass by 0.0623 Da or m/z of 0.0312. Separating these ions requires a mass resolution on the order of 25 000, again readily achievable by FTICR-MS (Figure 10a). FAIMS is also able to completely separate these ions as shown in the CV scans for these two ions shown in Figure 10c. Partial mass spectra obtained at the maximum in CV values for these two ions shown in Figure 10b clearly demonstrate the ability to completely separate these ions using FAIMS. It should be noted that these ions are only very minor components of the mass spectrum (~2.5% relative abundance) and no sodium was added to the sample. The sodium is present in the PEG 1500 as provided by the manufacturer. With increasing polymer size, separations of PEG ions that differ by the addition of two protons versus two sodium ions with one n -mer size smaller become increasingly difficult. FAIMS provides a method to potentially separate these species and would be particularly advantageous for lower resolution mass spectrometers.

CONCLUSIONS

By combining FAIMS with FTICR-MS, it is possible to significantly enhance the lower limit of detection, the dynamic range, and the detection sensitivity. These enhancements are primarily due to the ability of FAIMS to selectively introduce ions of interest into the FTICR-MS instrument. For ions of low abundance, this is particularly advantageous because these ions can be selectively accumulated while more abundant ions that can interfere with mass

detection performance due to adverse Coulomb interactions are reduced or even eliminated. Similar enhancements should occur when combining FAIMS with any ion storage instrument where ion-ion interactions reduce detection performance.

The signal enhancement observed with FAIMS depends on ion identity and the sample. Between 1.8- and 14-fold increases in signal are observed for various molecular ions of PEG 600. Equally important is the factor of 1.7 reduction in noise obtained using FAIMS due to the elimination of chemical noise. Mass spectra at any single FAIMS compensation voltage do not accurately reflect the distribution of ions formed by ESI, but this information can be accurately obtained from mass spectra acquired over a range of compensation voltages.

The compensation voltages for ion transmission do not directly correlate with polymer molecular weight, although collisional cross sections, as measured by drift tube ion mobility spectrometry, do correlate with polymer molecular weight. This demonstrates that the ion collisional cross section is not the most important parameter in the separation of these PEG ions using FAIMS. A strong correlation between polymer size necessary to solvate an attached cation versus cation size indicates that interactions between the carrier gas and the cations in these polymer ions play a major role in the separation of these ions using FAIMS. This is an important result because, in general, collisional cross section and ion mass are somewhat correlated. Because other factors that are not correlated with molecular size or mass influence ion transmission in FAIMS, FAIMS separations are more truly orthogonal to molecular weight than separation methods that are based solely on the collisional cross section. As with conventional ion mobility measurements done in a drift tube, different conformers that have the exact same mass can be separated using FAIMS. In addition, minor conformers that are less than 0.2% relative abundance can also be identified due to the high ion transmission possible with FAIMS.

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57. Previously measured collisional cross sections for these PEG ions are from refs ⁵⁰⁻⁵² and ⁵⁶. For a few specific *n*-mers, cross section data were not reported. In these cases, a value for the collisional cross section was estimated by linear interpolation between the collisional cross sections of PEG 600 ions with one fewer and one additional incorporated polymer subunit. These estimated values should have very little error in this range of *n*-mers due to the linear relationship between collisional cross section and number of incorporated polymer subunits.

Figure 1.
Schematic diagram of the FAIMS device attached to the ion entrance of the FTICR mass spectrometer.

Figure 2.

Relative intensities of singly charged sodiated PEG 600 ions: (a) maximum intensity through the FAIMS device observed for each PEG n -mer, (b) maximum intensity observed for each n -mer without FAIMS from a set of 10 mass spectra, (c) average relative intensity observed for each n -mer with FAIMS summed over ~ 10 spectra that contribute to a CV peak, and (d) average relative intensity observed for each n -mer without FAIMS (10-scan average). All relative intensities (a–d) are on the same scale. Note that the noise level on the 10-scan averages (c,d) are lower due to signal averaging. The enhanced ion abundances of PEG 7- and 10-mers in (c) are due to more selective transmission of these ions through the FAIMS device (see text).

Figure 3. Partial mass spectra of PEG 600 with FAIMS at compensation voltages of -7.4 (a) and -5.9 V (b). The ion intensities are plotted relative to the ion intensity in Figure 2a.

Figure 4.

Mass spectra and CV scan for singly charged sodiated PEG with 22 polymer subunits: mass spectra without (a) and with (b) the FAIMS separation and (c) the CV scan for this ion. The ion intensities are plotted relative to the ion intensity in Figure 2a.

Figure 5.

Compensation voltage scan of singly charged sodiated PEG with 14 polymer subunits: (a) a three-dimensional plot of CV versus partial mass spectra data around the region of the sodiated PEG 14-mer and (b) maximum relative conformer intensity versus CV. The major peak in transmission through the FAIMS device is labeled as region II. Minor peaks in ion transmission are labeled as regions I, III, and IV. The mass spectral data are not apodized so that the peak shape is Lorentzian. This results in broad peaks near the baseline (see green region in conformer II).

Figure 6. Major and minor peaks in ion transmission through the FAIMS device for singly charged sodiated PEG 600 with 5–27 polymer subunits. Smaller symbols correspond to low-abundance conformers.

Figure 7.

Major peaks in ion transmission through the FAIMS device for singly charged PEG 600: (a) maximum compensation voltage of ion transmission versus number of polymer subunits and (b) maximum compensation voltage versus measured collisional cross sections.⁵² Lines marked with diamonds, triangles, and squares correspond to (PEG + Li)⁺, (PEG + Na)⁺, and (PEG + Cs)⁺, respectively. Collisional cross sections for lithiated, sodiated, and cesiated PEG ions are from previously reported values measured by Bowers and coworkers.^{50-52,56,57}

Figure 8. Compensation voltage versus the number of incorporated polymer subunits for (PEG + 2Na)²⁺ (triangles), (PEG + Na + Cs)²⁺ (diamonds), and (PEG + 2Cs)²⁺ (squares), where the size of the symbol for each ion is proportional to the log of the absolute intensity for that ion.

Figure 9.

Compensation voltage and mass spectra data for $(\text{PEG}_{20} + 2\text{H})^{2+}$, $(\text{PEG}_{17} + \text{Cs} + \text{H})^{2+}$, and $(\text{PEG}_{19} + 2\text{Na})^{2+}$: (a) a three-dimensional plot of CV versus m/z data (b) an overlay of three mass spectra, one for each ion obtained at three different CV values, and (c) CV scans for each ion.

Figure 10.

Compensation voltage scan and mass spectra data for $(\text{PEG}_{34} + 2\text{H})^{2+}$ and $(\text{PEG}_{33} + 2\text{Na})^{2+}$ obtained from a PEG 1500 solution: (a) a three-dimensional plot of the CV versus m/z data (b) an overlay of two mass spectra, one for each ion obtained at two different CV values, and (c) an overlay of two CV scans, one for each ion.