Single Particle Analyzer of Mass: A Charge Detection Mass Spectrometer with a Multi-Detector Electrostatic Ion Trap

Andrew G. Elliott, Samuel I. Merenbloom, Satrajit Chakrabarty and Evan R. Williams*

Abstract

A new charge detection mass spectrometer that combines array detection and electrostatic ion trapping to repeatedly measure the masses of single ions is described. This instrument has four detector tubes inside an electrostatic ion trap with conical electrodes (cone trap) to provide multiple measurements of an ion on each pass through the trap resulting in a signal gain over a conventional trap with a single detection tube. Simulations of a cone trap and a dual ion mirror trap design indicate that more passes through the trap per unit time are possible with the latter. However, the cone trap has the advantages that ions entering up to 2 mm off the central axis of the trap are still trapped, the trapping time is less sensitive to the background pressure, and only a narrow range of energies are trapped so it can be used for energy selection. The capability of this instrument to obtain information about the molecular weight distributions of heterogeneous high molecular weight samples is demonstrated with 8 MDa polyethylene glycol (PEG) and 50 and 100 nm amine modified polystyrene nanoparticle samples. The measured mass distribution of the PEG sample is centered at 8 MDa. The size distribution obtained from mass measurements of the 100 nm nanoparticle sample is similar to the size distribution obtained from transmission electron microscopy (TEM) images, but most of the smaller nanoparticles observed in TEM images of the 50 nm nanoparticles do not reach a sufficiently high charge to trigger the trap on a single pass and be detected by the mass spectrometer. With the maximum trapping time set to 100 ms, the charge uncertainty is as low as ± 2 charges and the mass uncertainty is approximately 2% for PEG and polystyrene ions.

1. Introduction

Synthetic polymers are used in a wide variety of applications, from simple homopolymers used in common packaging materials and textiles [1,2], to carefully engineered copolymers and nanoparticles designed for electronics [3] and biomedical technologies [4-6]. Many properties of polymers are related to molecular weight [7], making accurate characterization of molecular weight distributions important to understanding and predicting polymer function. Soft ionization techniques, such as electrospray ionization (ESI) and matrix assisted laser desorption ionization (MALDI), can be used to produce gaseous ions of polymers even above 1 MDa molecular mass [8-10]. MALDI-MS can be particularly effective for mass analysis of large polymers because low charge states are typically produced, although differential ionization efficiency as a function of molecular weight can occur [11-13]. Careful matrix selection and sample preparation are important to effectively ionize MDa polymer molecules with MALDI [14-16], and cryogenic detectors have been used to overcome low detection efficiencies associated with the large mass but low charge state ions typically formed by MALDI at high mass to charge (m/z) values [15-18]. ESI has the advantage that highly charged ions are produced, which lowers their m/z to a range where the performance of many different types of mass spectrometers excel [19]. However, the charge state distributions of each molecular ion of a heterogeneous synthetic polymer sample with a broad molecular weight distribution can overlap, limiting the size of the largest synthetic polymer than can be analyzed using ESI with

conventional MS instruments [20]. A rough measure of the molecular weight of large biopolymers formed from solutions in which they have native-like spherical structures can be obtained from the m/z of the unresolved charge state distribution, but with significantly less accuracy than if the charge states can be determined from the mass spectral data.

An alternative way to obtain molecular weight information from a heterogeneous sample consisting of large molecules is to form and weigh individual ions so that the signal from two ions cannot overlap. There are several different methods to weigh individual ions. Quadrupole ion trap (QIT) instruments can trap single ions for extended periods of time, enabling high precision m/z measurements by observing the motions of the ion in the trap through light scattering or fluorescence [21-26]. The mass can be determined by making repeated m/z measurements after changing the charge of the ion. Single highly charged ions have also been measured with Fourier transform ion cyclotron resonance (FT-ICR) [27-31] and Orbitrap [32,33] instruments. In FT-ICR instruments, the charge state of each ion can be obtained by measuring the ion multiple times after changing its charge, just like with the QIT [27-29]. The signal intensity induced by a single ion can also provide information about its charge state in both FT-MS techniques. However, precisely measuring the charge of a single ion from the induced current can be challenging because the amplitude of the induced current depends on the radial position of the ion in the detector as well as its charge [34,35]. To account for this effect on single ion FT-ICR-MS, Smith and coworkers measured individual ions multiple times, exciting each ion to a larger and larger radius so that charge measurements could be made from a single known radius corresponding to a value slightly less than that of the cell [30,31]. Because each ion must be measured several times to accurately determine its charge state, weighing an ion

with these single ion detection techniques can be quite slow. The high mass precision possible with the QIT, FT-ICR or Orbitrap comes at a cost of long analysis times (many seconds to minutes) for each ion.

A faster way to measure the mass of single ions is with charge detection mass spectrometry (CDMS). Shelton and coworkers first demonstrated CDMS over 50 years ago, measuring the mass of micron sized iron particles with 10^4 charges by passing individual ions through a single conducting cylinder [36]. As each ion passes through the conductor, it induces an image charge that is proportional to the charge of the ion. The velocity of the individual ion is obtained from the tube length and from the duration of the image charge signal corresponding to the ion entering and exiting the tube. The m/z of the ion is determined from its energy and velocity. The mass of each ion is obtained from the independent charge and m/z measurements. The m/z and charge of each ion are measured simultaneously in the few microseconds that it takes for the ion to pass through the detector. Thus, each ion can in principle be weighed quickly. Ions with the same m/z but different charge states are readily distinguished from each other because each ion is weighed individually. A true mass spectrum, versus the more conventionally measured m/z spectrum, is obtained for all samples as a histogram of all of the measured individual masses.

Single pass CDMS instruments similar to the design of Shelton and coworkers have been used to measure the mass of many different types of particles. Early experiments with CDMS focused on small cosmic dust particles, and instrumentation advancements resulted in improved detection limits down to 1500 charges [37-39]. Fuerstenau and coworkers expanded single pass

CDMS to biomolecules and synthetic polymers and measured the mass of plasmid DNA [40] and polystyrene spheres [40], as well as tobacco mosaic virus and rice yellow mottle virus [41]. Jarrold and coworkers observed negatively charged droplets from positive electrospray with single pass CDMS [42]. Dugourd and coworkers also used single pass CDMS to study synthetic polymers and nanoparticles over a wide size range, from low MDa polyethylene glycol (PEG) ions up to composite nanoparticles weighing tens of GDa [43-47]. In these instruments, single ions with as few as 300 charges were detected, although large uncertainties in measured m/z and charge values limited the overall mass precision of these measurements.

The uncertainty in CDMS measurements can be improved by signal averaging repeated measurements of the same ion. One approach is to use multiple detector tubes and measure the ion as it passes through each tube. This linear array of detector tubes improves the measurement uncertainty by a factor of $n^{1/2}$ where n is the number of tubes in the array. Gamero-Castaño first used this method to study micron-sized propylene carbonate droplets with a detector consisting of six cylinders [48]. The longest array detector used for CDMS thus far contained 22 detection tubes, with which Jarrold and coworkers demonstrated a charge uncertainty as low as ± 9 charges [49]. Austin and coworkers developed a detector array on a printed circuit board, simplifying the design and construction of a long array detector [50]. However, a limitation of array detectors is that the detection capacitance increases with the number of detectors, which decreases the charge detection sensitivity.

Another approach to detecting an ion multiple times is to trap the ion and make it pass through the charge detection region multiple times. Dual ion mirror traps have been used to

measure the m/z of ensembles of small ions with resolution up to $\sim 10^5$ [51-55]. Benner demonstrated the use of a dual ion mirror trap for single particle CDMS with a single detector tube [56]. The ion mirror at the trap entrance is initially at ground potential until a signal pulse from an ion is detected. This signal triggers an increase in the entrance mirror potential to close the trap. A minimum of ~ 250 charges on an ion was necessary in order for there to be a detectable signal that would result in a trap closure. Ions were trapped for up to 450 cycles and an uncertainty in the charge measurement of single DNA ions of ± 2.3 charges was reported. Dugourd and coworkers demonstrated photodissociation of individual mass selected PEG and DNA ions inside a similar ion mirror trap [57-59].

Jarrold and coworkers used Fourier transforms to analyze CDMS data to obtain masses of single ions with as few as 7 charges [60]. Because a low charge ion produces an insufficient signal to induce a trapping event, data were obtained by raising the potential of the entrance electrode without knowing if an ion is present in the detector [61]. A Fourier transform of the resulting time domain signal from a single trapped ion results in a frequency related to the m/z of the ion and amplitude that is proportional to the charge of the ion. With cryogenic cooling of the detector [62] and a trapping time of 6×10^4 cycles over 3 s [63], they demonstrated an uncertainty of ± 0.196 charges with fewer than 1 in 10^4 ions assigned to the wrong charge state. This FT-CDMS approach has extended the range of samples that can be investigated with CDMS to include small proteins, and has improved the resolution and mass measuring accuracy for large virus particles by CDMS [60-67].

Here, we describe a new CDMS detector consisting of an array of four charge detection tubes inside an ion trap, that we refer to as the single particle analyzer of mass (SPAM). By combining both trapping and array detector approaches, ions can be trapped for hundreds of passes, and more measurements per unit time can be made because ions spend a larger fraction of time inside a detector tube. Simulations show the cone trap design developed by Schmidt et al. [68] has characteristics that are advantageous for CDMS. The performance of the instrument is tested with complex polymer and nanoparticle samples and implications for the reported size dependence of PEG ionization mechanisms are discussed.

2. Experimental

2.1. Samples. Polyethylene glycol (PEG) with a nominal molecular weight of 8 MDa and amine-modified polystyrene nanoparticles with nominal diameters of 50 nm (~41 MDa) and 100 nm (~330 MDa) were obtained from Sigma Aldrich (St Louis, MO). The PEG samples were prepared at a concentration of 0.1 mg/mL in a 50/50 water/methanol solution. The nanoparticle samples were prepared at 0.125% in a 75/25 methanol/water solution. Electron microscope images of nanoparticles were acquired on a Tecnai 12 transmission electron microscope (FEI, Hillsboro, OR). One drop of the sample solution was deposited on a copper grid for each sample. The diameters of the particles were measured from the images using Adobe Illustrator.

2.2. Mass Spectrometry. Experiments were performed on the single particle analyzer of mass (SPAM), a home-built charge detection mass spectrometer, a schematic of which is shown in Figure 1. Ions are formed by nanoelectrospray ionization using borosilicate capillaries that

have tips pulled to an inner diameter of between 2 and 60 μm . A platinum wire is inserted into the capillary so as to be in contact with the sample solution. A potential of +1-2 kV with respect to instrument ground is applied to the capillary to initiate ion formation by electrospray. The resulting ions are transferred to vacuum using a Z-spray source (Waters Corporation, Milford, MA) modified to contain an extraction cone with a 0.5 mm hole to improve differential pumping between the source and first vacuum stage. Typical voltages inside the source are +335-450 V for the exterior sample cone and +205 V for the interior extraction cone and the pressure between the cones is typically 1 Torr. The source is heated to 80-90 $^{\circ}\text{C}$ to improve ion desolvation. After the extraction cone, ions enter the first vacuum stage at a pressure of 5×10^{-4} Torr then pass into a second chamber at 5×10^{-6} Torr through a grounded skimmer. A single einzel lens consisting of three stainless steel tubes guides ions to the detection chamber through a 1.0 mm conductance limit where they can be trapped at a pressure of 1×10^{-8} Torr. Transmission of large nanoparticles was improved by raising the pressure in the source block to 70 Torr by partially closing a valve to the source pump. The resulting pressures were 2×10^{-3} Torr, 2×10^{-5} Torr, and 3×10^{-8} Torr in the first, second and third chambers, respectively.

The detection system consists of four stainless steel tubes that are between two conical trapping electrodes that are based on the design of Schmidt et al. [68]. The tubes have an inner diameter (ID) of 7.1 mm and are 28.2 mm long. Each tube is held in place by insulators inside a 31.8 mm ID shielding tube, and the total distance separating each trapping electrode is 127 mm. The four tubes are connected pairwise in the pattern shown in Figure 1b, with tubes 1 and 3 connected to one detection circuit and tubes 2 and 4 connected to a second circuit. Each pair of tubes is connected to a separate CoolFET charge sensitive preamplifier (Amptek, Bedford, MA)

containing an A250 preamplifier and JFET cooled to $-50\text{ }^{\circ}\text{C}$ by a Peltier cooler. The preamps are housed in a shielded box inside the vacuum chamber, and the signal is sent to a shaping amplifier consisting of a differentiator, integrator and two voltage amplifiers (Amptek A275) and signal filter outside the vacuum chamber. Data for both channels are recorded simultaneously with a single analog to digital converter card (ATS9350, AlazarTech, Pointe-Claire, Canada) at a rate of 2 MHz. The preamplifier circuit for the A channel (tubes 1 and 3) has a 1 pF test capacitor composed of ten 10 pF 1% capacitors. To calibrate the detector, a charge pulse of known amplitude was passed through the test capacitor into the amplifier system. The charge response on the B channel (tubes 2 and 4) was later calibrated against the charge measured with signal from channel A by comparing trapped ions measured at less than 3% uncertainty in the charge and amplitude measurements from channel A and B, respectively. The response from the first and second channels is $140\text{ }\mu\text{V/charge}$ and $62\text{ }\mu\text{V/charge}$, respectively. The noise on the second channel is correspondingly lower such that both channels have similar signal-to-noise (S/N) ratios.

An ion entering a detector tube induces an image charge of opposite sign on the tube. Inverting and shaping the square wave signal from that tube produces a pattern consisting of a leading positive peak and trailing negative peak for a positive ion. The spacing between the peaks is proportional to the velocity of the ion. If the rise time of the image charge pulse is shorter than the peaking time of the shaping amplifier, the peak widths are constant and the amplitude of each peak is proportional to the charge of the ion. The peak widths of the PEG and nanoparticle ions are comparable to the peak width from the calibration with the test capacitor, which indicates that the peak amplitude provides a good estimate of the ion charge over the size

range of the ions reported here. The standard deviation of charge measurements from an ion entering or exiting a single tube is ± 105 charges, or about 10% of the average charge on an 8 MDa PEG ion. The relative standard deviation of single tube velocity measurements is 3.7%. Repeated measurements of these values for a single ion decreases the associated uncertainty by a factor of $n^{1/2}$, where n is the number of measurements.

Two different ranges of ion energies can be trapped at a single trapping potential on the cone trap, with a narrow range near the trap potential and wider range at $\sim 60\%$ of the trap potential [68]. In these experiments, the ion trap is only operated in the upper energy range in order to obtain energy selection. The cone electrode in the back of the trap is maintained at a single trapping potential for the entire experiment, whereas the voltage on the front is initially lower to allow ions to enter the trap. Upon sensing a charge pulse from a detector tube greater than a specified amplitude, the voltage on the front of the trap is pulsed up to the same voltage as the back of the trap through a MOSFET switch and reaches the trapping potential 20 μs after the detection of the charge pulse. For the samples studied here, trapped ions typically take at least 100 μs to return to the first cone electrode, so no ions are lost before the trap is closed. The front of the trap is held at the trapping potential for 100 ms corresponding to the time of the data acquisition. After the measurement, the front voltage is lowered for 8 ms and the acquisition program is reset in order that the trap can trigger on the next ion detected in the trap. The upper limit of the trap cycle is 9 Hz.

Data is analyzed with a set of LABVIEW programs. Signals from trapped ions are first sorted from transients in which the detected ion was not trapped by checking for ion signal

corresponding to each pass of an ion's second cycle through the trap. To account for different size ions taking different amounts of time to turn around, ion signal was identified by comparing the maximum and minimum values of the root mean square of a time interval stepped through the expected time of each pass through the detector. A different time interval was chosen for each sample, approximately equal to the time for a typical ion to pass through the detector or turn around. For each transient that contains an ion, peaks corresponding to the ion entering and leaving each detector tube are identified with a peak picking routine designed to identify signal from low charge ions based on the consistent amplitude and spacing between induced peaks. The average peak spacing and approximate peak height are determined from the group of four peaks containing the largest peak in the transient. These peaks correspond to a single pass through the detector array. Then the entire transient is scanned for groups of four peaks close to the amplitude of the initial set of peaks that are spaced at the same time interval as the initial set of peaks. With the array of peak timings and amplitudes, the m/z , charge and mass can be determined using the charge calibration described above. The m/z of the ion is proportional to the measured velocity and trapping voltage from Eq. 1:

$$, \quad (1)$$

where V is the trapping potential, v is the ion velocity and C is a proportionality constant to convert from the trapping potential to the ion energy. A C value of 0.96 was determined from the range of ion energies that are trapped at a given potential using SIMION simulations. Because only ions within a narrow range of kinetic energies are trapped, there is no need for specific terms in Eq. 1 to account for aerodynamic acceleration.

3. Results and Discussion

3.1. Ion Trap Design. Two electrode designs have been used in trapping CDMS instruments: dual ion mirror traps [52,56,57] and cone traps [61,68]. In order to compare the performance features of the two designs and evaluate the effects of various parameters, ion trajectory simulations were performed on the two designs in SIMION 8.0 using ions weighing 17 kDa with 21 charges for all simulations. The time for one complete cycle for a trapped ion of the same mass, kinetic energy, and charge state differs for the two traps. This effect is illustrated for an ion with a kinetic energy of 230 eV/charge in Figure 2a, which shows the location of the ion along the trap axis as a function of time. In the cone trap, shown in the upper trajectory, the ion travels far beyond the detector tube, penetrating deep into the electrode and takes 56 μ s to return to the tube. In the ion mirror trap, shown in the lower trajectory, the ion turns around much closer to the entrance of the ion mirror, and spends only 9.3 μ s outside the tube on each pass. Ions turn around faster and pass through the detector tubes more times for a given trap time with the ion mirror trap. Approximately three fold more measurements can be made with an ion mirror trap than a cone trap for a given trap time under these conditions. The effect of ion kinetic energy on the ion trapping time at a given trapping potential was evaluated at a trapping potential of 240 V, with a maximum trapping time set at 50 ms. These data (Figure 2b) show that only ions with an energy between 88% and 94% and between 96% and 98% of the trapping potential are efficiently trapped in the ion mirror trap and cone trap, respectively. The range of energies at which an ion is trapped at a given potential is much narrower for a cone trap, with a 2% range in the ion energy

compared to 6% for the ion mirror trap. Thus, a cone trap can be used as an energy filter for the trapped ion. This narrow range of energies for successful ion trapping reduces the uncertainty in the m/z measurement for each ion in the absence of other energy filters prior to the trap.

The ion trapping time also depends on the initial radial position and the angle at which an ion enters the trap [56,57]. The effect of the starting position on the trapping time of an ion in each trap was investigated with ions starting in a direction parallel to the trap axis, but up to 3 mm off center. The fraction of the 50 ms maximum trapping time the ion remains in the trap is shown as a function of the distance from the center of the trap axis in Figure 2c. The acceptance diameter for the cone trap is 3.5 mm, which is about two times greater than that of the ion mirror trap. To determine the effects of pressure and angular dispersion on trapping time, ion trajectories starting on center but with initial trajectories up to 1.4° off the trap axis were simulated at 10^{-5} to 10^{-7} Torr using a hard sphere collision model in SIMION (Figure 2d). Ions entering up to 0.2° off center were trapped for similar lengths of time in each trap, but beyond that angle, ions in the ion mirror trap were quickly lost from the trap at each pressure tested. Trapping times in the cone trap are greater than 1 ms at pressures up to 10^{-6} Torr at entrance angles up to 1.0° . In addition to better energy selection features, the cone trap also has greater tolerance for ions entering the trap on trajectories off its central axis.

3.2. Mass Measurement of a Single Ion. To illustrate the mass measurement of a single ion with sufficient charge to induce an observable signal upon a single pass through each detection tube, a transient signal from each of the two detector channels for a single ion obtained from a sample of 100 nm polystyrene beads is shown in Figure 3. Each time an ion passes

through the detector tubes, it induces eight voltage pulses corresponding to each time the ion enters or exits a single tube, with four pulses on each detection channel. Peaks A and B on the upper trace (channel A) are the result of the ion entering and exiting the first detector tube, respectively. As the ion enters the second tube, it then induces peak C on the lower trace (channel B), and peak D as it leaves the second tube. Peaks E and F correspond to the ion in the third detector tube, and peaks G and H the ion in the fourth tube. After the ion turns around, it induces the same pulse pattern on its way back through the detector tubes, with points A' and B' from tube four, C' and D' from tube three, E' and F' from tube two, and G' and H' from tube one, in a repeating pattern until the ion is lost from the trap. This ion was trapped for 63 ms, during which time it traveled 56 cycles (112 passes) through the trap at a velocity of 562 m/s in the field free region of the trap and had 624 ± 4 charges. The trap potential was 340 V, corresponding to a kinetic energy of 327 eV/charge. Thus, the m/z of this ion is $199,000 \pm 4,000$ and its mass is 124 ± 3 MDa.

The mass measurements made with each set of tubes are similar for each ion despite the greater gain in channel A for tubes one and three. For the ion shown in Fig. 3, the charges and masses obtained from the two separately measured channels are 622 ± 5 charges and 124 ± 3 MDa for tubes one and three and 625 ± 6 charges and 125 ± 3 MDa for tubes two and four. These charge uncertainties correspond to the standard deviation of the mean of the charge measurements. With four charge measurements per pass and 112 passes, the charge of this ion was measured 448 times on each channel, which decreases the uncertainty by a factor of 21, from ± 105 charges for a single measurement to ± 5 charges for the entire measurement. For 701

ions that were trapped for more than 2 cycles through the trap, a correlation plot of the mass obtained from each channel produces a best fit line with a slope of 0.9974 and an R^2 of 0.9705.

3.3. Trap Performance. It is essential to trap ions for an extended period of time in order to make precise mass and charge measurements. Only ions within a narrow range of energies are trapped which provides energy selection. Only ions that complete at least two cycles are used for determining the masses of individual molecules in each sample, which is approximately 1% of all ions entering the detector array in the present configuration. The uncertainty in the charge and velocity measurement decreases with an increasing number of passes through a detector tube. The distribution of trap residence times and corresponding charge uncertainties from a single detection channel for the 376 trapped ions from an 8 MDa PEG sample (Figure 4) illustrates the advantages of a longer trapping time to improve the precision of the charge measurement. The uncertainty in the measured charge is 1% for ions that are trapped for more than 10 ms or 30 cycles, which represents approximately 20% of the trapped ions. Approximately 10% of the ions are trapped for at least 30 ms (100 cycles), at which point, the charge uncertainty is ± 3 charges. Jarrold and coworkers have reported CDMS measurements with lower charge uncertainties of ± 2 charges for protein ions trapped for 30 ms [60], and as low as ± 0.196 charges for a trapping time of 3 seconds [63]. However, smaller ions travel faster and can complete more cycles in a given time, resulting in more measurements and lower uncertainty in the charge measurement. For ions trapped for 400 tube transits (equivalent to the signal induced on one channel in 100 cycles in our detector), the charge uncertainty reported by Jarrold and coworkers is approximately ± 3 charges [64], which is similar to the results reported here for 8 MDa PEG.

The trapping time for different mass ions was investigated using 50 and 100 nm polystyrene beads, and 8 MDa PEG. Approximately 10% of the trapped nanoparticles remained trapped for the entire 100 ms duration of each trapping event, but just one PEG ion out of 376 was trapped for that length of time. However, the 100 nm polystyrene spheres are much larger and travel slower than the PEG ions, resulting in a time per cycle that is on average three times longer. Because of this longer cycle time, no ions from the sample of 100 nm polystyrene spheres were trapped for more than 120 cycles in the 100 ms, which is similar to the number of cycles PEG ions made in 30 ms. About 10% of ions from all three samples were trapped for approximately 100 cycles indicating that the trapping efficiency for a given number of cycles does not depend significantly on the molecular weight within this range. The charge uncertainties for the nanoparticles are around ± 2 charges for ions trapped for the longest times, which is similar to that obtained for the smaller PEG ions in the same number of cycles.

3.4. Polyethylene Glycol. In order to obtain useful information about the distribution of molecular weights of the many compounds that may be present at different concentrations in a complex mixture, it is necessary to weigh many ions individually. For a more uniform sample, fewer ions are necessary to provide useful information. Mass information about an 8 MDa PEG sample was obtained by weighing 376 individual ions, and a mass spectrum that is made up from a histogram of the individual masses is shown in Figure 5. The distribution has a maximum around 7-9 MDa with a full-width-half-maximum (FWHM) of 6 MDa, and a high mass tail that extends up to 39 MDa. There are also peaks apparent at 14 and 25 MDa. The maximum in this mass distribution is about the same as the reported average molecular weight, and the peaks at 14 and 25 MDa likely correspond to dimers and trimers. The dispersity index calculated from the

measured masses is 1.4, consistent with reported dispersity indexes for MDa PEGs which range from 1.4 to 2.8 [69,70]. Approximately half of the ions have between 600 and 1000 charges, with a maximum of about 1100 charges for an ion at 8 MDa and up to 2900 charges for the largest ions trapped (~40 MDa). As a result, the m/z spectrum is centered at ~10,000 with only three ions below $m/z = 6,000$.

These mass measurements are similar to those reported by Dugourd and coworkers who used CDMS to measure the molecular weight of a 7 MDa polyethylene glycol sample [43]. They found that the molecular weight was near the expected value and had a similarly broad distribution of 7 MDa FWHM. This broad peak was attributed to the inherent dispersity in the sample, which is the likely cause of the broad range of masses in both measurements. They also reported that the 7 MDa PEG ions charged to a similar level as what is reported here.

3.5. Ionization Mechanism of Polyethylene Glycol. Fenn and coworkers reported ESI mass spectra of PEGs between 400 Da and 5 MDa and used the m/z of the ions together with a modeled charge capacity to deduce mechanistic information about how PEG ions are formed by ESI [71,72]. In their model, PEG ions theoretically reach a maximum charge state in a linear zigzag conformation, with charges evenly spaced along the entire length of the ion. The maximum charge state decreased with increasing ion mass up to 20-30 kDa relative to the maximum charge for a theoretical linear ion. For PEG samples weighing more than 17.5 kDa, they observed a broad peak between m/z 600 and 1200 which they assigned to intact PEG molecular ions. The maximum reported charge state for these larger ions was 4200 charges for 5 MDa PEG, and the reported maximum charge for each ion increased relative to the modeled

maximum charge with increasing mass. Based on these data, they concluded PEG molecules smaller than 20-30 kDa are ionized by an ion evaporation mechanism, whereas larger PEG ions are instead formed by a charge residue mechanism necessary in order to reach high charge states.

The maximum charge states for MDa PEG ions reported by Fenn and coworkers [71,72] are significantly higher than those measured with CDMS reported here and by Dugourd and coworkers [43,57]. None of the mass spectra for PEGs beyond 3.3 kDa measured previously by Fenn and coworkers had peaks with a resolvable charge state distribution with which the mass or charge of those ions could be determined. The quadrupole mass spectrometer used in the earlier experiments had a m/z limit of 1500, well below where PEG molecular ions are observed in our experiments. Although there may be some difference in the extent of charging in these experiments owing to differences in solution or instrument conditions, our data, which show much lower charging for large, intact PEG molecules, indicate that the ions reported by Fenn and coworkers to be intact large PEG molecules are more likely to be smaller fragments. Thus, our data bring into question the mechanistic conclusions about the size dependence of the ion formation mechanisms deduced from this prior data. It is important to note that the Rayleigh limit charge for a spherical 8 MDa water droplet is 223 charges, a value much lower than the charge states observed here and by Dugourd and coworkers. This indicates that the ions in our experiments are unfolded to a large extent in the ESI droplets prior to ion formation.

3.6. Amine-modified Polystyrene Nanoparticles. To evaluate the accuracy with which information about the molecular weights of larger, more complex samples can be obtained, samples of 50 and 100 nm amine modified polystyrene nanoparticles were measured. The mass

spectrum of the 50 nm sample obtained by CDMS (Figure 6a) has a maximum at 35 MDa, with a FWHM of 6 MDa. The approximate sizes of the ions measured in SPAM can be obtained from these mass measurements by assuming these particles are spherical with a density of 1.05 g/mL (value reported by the manufacturer). Figure 6b shows the mass spectrum converted into a size distribution, with a maximum at 48 nm, near the manufacturer stated size of the sample. To obtain independent information about the size distribution, the nanoparticle sample was imaged using transmission electron microscopy (TEM) (Figure 6d). The size distribution measured from those images (Figure 6c) shows a broad range of particle sizes peaking at 40 nm. In contrast, no ions smaller than 43 nm were detected by CDMS.

The larger particle distribution obtained by MS is an artifact of how the CDMS measurement was made. To ensure the trap only closed when an ion was present, the trap was set to operate in a triggering mode, in which ions are only trapped when they induced a signal corresponding to at least ~575 charges on their first pass through the detector. Ions with fewer than 575 charges are still trapped when constructive interference between ion signal and noise is above the trigger limit, but few ions with less than 400 charges were trapped. The measured charge of the trapped ions and the Rayleigh limit charge for a water droplet as a function of size are shown in Figure 7. Most of the ions have charge that is close to, but slightly less than the Rayleigh limit, with an average value of 92% of the Rayleigh limit charge. The Rayleigh limit charge for a 45 nm nanoparticle is 425, which is close to the minimum necessary to occasionally trigger a trapping event. This threshold for trapping results in the majority of ions smaller than 46 nm not being detected. Improvements in the detector electronics or cooling to liquid nitrogen temperatures would improve the detection limit. The random trapping method used by Jarrold

and coworkers can also be used to detect ions with as few as 7 charges [60], but this method of data acquisition is less efficient because some trapping events occur when there are no ions or multiple ions present.

In order to more fully evaluate the mass accuracy of the instrument, measurements were made on a sample of 100 nm amine-modified polystyrene beads that should be sufficiently large to consistently charge above the trigger threshold. The CDMS mass spectrum and resulting size distribution of this sample are shown in Figure 8a and 8b, respectively. The sample contains a broad range of particle sizes, with a maximum between 66 and 76 nm, and an average size of 78 nm. The particle sizes measured by CDMS are much smaller than that indicated by the manufacturer. The distribution measured from TEM images (Figure 8c and d) is also broad, with a wide peak stretching from 60 to 80 nm and an average size of 70 nm. The CDMS and TEM data show a similar distribution of sizes, but the average size from CDMS measurements is slightly higher. Salt or solvent adduction may increase the particle masses measured by CDMS, shifting the size distribution larger than that of the bare particles in the TEM images. The size distribution measured from TEM images may also be slightly lower than the true distribution due to uncertainties in determining the edges of the nanoparticles in the images. Uncertainty in the density used to obtain the nanoparticle diameter from the CDMS mass measurements may also contribute to the differences between the two measured size distributions.

3.7. Effects of Aerodynamic Acceleration on Ion Energy. Another potential source of uncertainty in measuring the mass of a single ion is aerodynamic acceleration from the gas expansion that takes place in the ESI source. In molecular beams of small molecules, dilute gases

accelerate to approach the speed of the background gas, with some velocity slip [73]. Large ions can also travel near the velocity of the neutral gas in an expansion, which increases the ion kinetic energy above that provided by electrostatic acceleration alone. Fuerstenau and Benner reported this effect for 314 nm polystyrene nanoparticles for which the initial velocity in the expansion was as much as 90% of the velocity measured in the detector tube of a single pass CDMS instrument [40]. In contrast, the expansion velocity was just 10% of the measured velocity for smaller DNA and PEG ions. If this initial velocity is not considered, the mass measurements would be lower than the actual mass of the particle. To account for aerodynamic acceleration, Dugourd and coworkers measured the mass of several standard sized nanoparticles to create a calibration curve between measured and actual particle masses [45,46].

In the experiments reported here, a narrow range of ion kinetic energies is selected using the trap potential. In order to investigate the effect of aerodynamic acceleration on the mass measurement made at a single trap potential, mass spectra of the 100 nm polystyrene bead sample were obtained at different trap potentials. The size distribution measured at each trapping potential is shown in Figure 9. The masses of the ions that are trapped increase with increasing trap potentials. At the lowest potential (300 V), no ions larger than 61 nm were trapped. In contrast, no ions smaller than 78 nm were trapped at the highest potential (390 V). At each trapping potential, only a 6-8 eV/charge range of ion energies is trapped. However, the range of ion energies is nearly 100 eV/charge. Thus, just a fraction of the entire ion distribution is measured at a single trap potential, and the mass and particle kinetic energy are related.

At a given velocity, ion kinetic energy is directly proportional to mass. However, for large globular ions like these nanoparticles, charging and thus the kinetic energy per charge both increase proportionally to the square root of the mass of the ion [74]. A 90 nm ion has approximately six times the mass of a 50 nm ion and therefore has approximately six times the kinetic energy if both ions have the same velocity, but just 2.4 times the charge. An average particle size of 51 nm and 92 nm is trapped at 300 V (Figure 9a) and 380 V (Figure 9i), respectively. With an electrostatic acceleration of 200 eV/charge, the ions trapped at 300 V have 86 eV/charge initial kinetic energy, and the ions trapped at 380 V have 163 eV/charge initial kinetic energy, 1.9 times more than the smaller particles. This ratio is close to, but less than, the energy per charge difference if the two ions were accelerated to the same initial velocity. This is consistent with the velocity slip increasing with particle size during aerodynamic acceleration.

Due to these energy effects of aerodynamic acceleration, the range of masses that can be measured is heavily influenced by the trap potential. For complex samples containing a broad range of particle sizes or masses, using many trapping voltages is important to effectively measuring the sample distribution. In the future, trapping and collisionally cooling ions before they are introduced into the trap, or increasing collisional cooling of the ions after the ESI source could be used to decouple the effects of aerodynamic acceleration from the ion energy in these trapping experiments.

4. Conclusions

A new charge detection mass spectrometer combines a four tube detector array within a cone trap. Using multiple detector tubes compensates for the slower turning time for the cone trap, so that a similar number of measurements are made in a cone trap and an ion mirror trap with a single detector tube in the same length of time for ions with the same m/z . Simulations show that the cone trap has greater tolerance for ions entering the trap off the central axis, can operate at higher pressures, and is more effective for energy selection than the ion mirror trap. The charge measurement uncertainty with this instrument is ± 3 charges and ± 2 charges for 8 MDa PEG ions trapped for 30 ms and 100 ms, respectively. These charge uncertainties are similar to those reported by Jarrold and coworkers for a similar number of measurements [64], but somewhat higher than those reported for a similar measurement time [60] due to the higher m/z and lower velocity of the high mass PEG ions.

The mass distribution of 8 MDa PEG is centered near the nominal mass of the sample and has a dispersity consistent with measurements made with other analytical techniques [69,70]. The directly measured charges of intact 8 MDa PEG ions are much lower than previously reported for large PEG ions where charge state resolution was not achieved [72], bringing into question mechanistic information about ion formation deduced from the earlier data. The size distribution obtained from mass measurements of 100 nm polystyrene nanoparticles indicates the nanoparticles are smaller than reported by the manufacturer, which is consistent with data from TEM images. The mass uncertainty of the 8 MDa PEG and polystyrene nanoparticle ions is approximately 2% and does not depend on mass within this size range. These results indicate single ion CDMS is a useful method for measuring molecular weight distributions of large, heterogeneous, polymer samples. These results also show that aerodynamic acceleration can

adversely affect mass measurements when there is a wide range of masses. This can be overcome by trapping or collisionally cooling ions prior to their injection into the trap. Addition of an ion energy filter will decrease the uncertainty in ion energy and will lead to more precise mass measurements.

Acknowledgements

The authors thank Waters Corporation for their generous donation of equipment, including the Z-spray source used in this work, the Robert D. Ogg Electron Microscope Lab at the University of California, Berkeley for use of the Tecnai 12 transmission electron microscope, and the National Institutes of Health (R01GM096097 and Grant 5F32GM093593-02 for fellowship support for S.I.M) and the Swiss National Science Foundation (Grant P2BSP2_148624 for fellowship support for S.C.) for funding.

References

- [1] V. Siracusa, P. Rocculi, S. Romani, M.D. Rosa, Biodegradable polymers for food packaging: A review, *Trends Food Sci. Technol.* 19 (2008) 634-643.
- [2] G.Q. Chen, M.K. Patel, Plastics derived from biological sources: Present and future: A technical and environmental review, *Chem. Rev.* 112 (2012) 2082-2099.
- [3] J.K. Kim, S.Y. Yang, Y. Lee, Y. Kim, Functional nanomaterials based on block copolymer self-assembly, *Prog. Polym. Sci.* 35 (2010) 1325-1349.
- [4] A. Rösler, G.W.M. Vandermeulen, H. Klok, Advanced drug delivery devices via self-assembly of amphiphilic block copolymers, *Adv. Drug Deliv. Rev.* 64 (2012) 270-279.
- [5] Z.L. Tyrrell, Y. Shen, M. Radosz, Fabrication of micellar nanoparticles for drug delivery through the self-assembly of block copolymers, *Prog. Polym. Sci.* 35 (2010) 1128-1143.
- [6] P. Alexandridis, Amphiphilic copolymers and their applications, *Curr. Opin. Colloid Interface Sci.* 1 (1996) 490-501.

- [7] A. Albanese, P.S. Tang, W.C. Chan, The effect of nanoparticle size, shape, and surface chemistry on biological systems, *Annu. Rev. Biomed. Eng.* 14 (2012) 1-16.
- [8] S.D. Hanton, Mass spectrometry of polymers and polymer surfaces, *Chem. Rev.* 101 (2001) 527-570.
- [9] T. Gruending, S. Weidner, J. Falkenhagen, C. Barner-Kowollik, Mass spectrometry in polymer chemistry: A state-of-the-art up-date, *Polym. Chem.* 1 (2010) 599-617.
- [10] L. Charles, MALDI of synthetic polymers with labile end-groups, *Mass Spectrom. Rev.* 33 (2014) 523-543.
- [11] P. Lloyd, K. Duddaby, J. Varney, E. Scrivener, P. Derrick, D. Haddleton, A comparison between matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry and size exclusion chromatography in the mass characterisation of synthetic polymers with narrow molecular-mass distributions: Poly(methyl methacrylate) and poly(styrene), *Eur. J. Mass Spectrom.* 1 (1995) 293-300.
- [12] K. Martin, J. Spickermann, H.J. Rader, K. Mullen, Why does matrix-assisted laser desorption/ionization time-of-flight mass spectrometry give incorrect results for broad polymer distributions? *Rapid Commun. Mass Spectrom.* 10 (1996) 1471-1474.
- [13] N. Sakurada, T. Fukuo, R. Arakawa, K. Ute, K. Hatada, Characterization of poly (methyl methacrylate) by matrix-assisted laser desorption/ionization mass spectrometry. a comparison with supercritical fluid chromatography and gel permeation chromatography, *Rapid Commun. Mass Spectrom.* 12 (1998) 1895-1898.
- [14] D.C. Schriemer, L. Li, Detection of high molecular weight narrow polydisperse polymers up to 1.5 million daltons by MALDI mass spectrometry, *Anal. Chem.* 68 (1996) 2721-2725.
- [15] R.J. Wenzel, U. Matter, L. Schultheis, R. Zenobi, Analysis of megadalton ions using cryodetection MALDI time-of-flight mass spectrometry, *Anal. Chem.* 77 (2005) 4329-4337.
- [16] A.A. Aksenov, M.E. Bier, The analysis of polystyrene and polystyrene aggregates into the mega dalton mass range by cryodetection MALDI TOF MS, *J. Am. Soc. Mass Spectrom.* 19 (2008) 219-230.
- [17] X.Y. Chen, M.S. Westphall, L.M. Smith, Mass spectrometric analysis of DNA mixtures: Instrumental effects responsible for decreased sensitivity with increasing mass, *Anal. Chem.* 75 (2003) 5944-5952.
- [18] L.D. Plath, A. Ozdemir, A.A. Aksenov, M.E. Bier, Determination of iron content and dispersity of intact ferritin by superconducting tunnel junction cryodetection mass spectrometry, *Anal. Chem.* 87 (2015) 8985-8993.
- [19] J. Snijder, R.J. Rose, D. Veessler, J.E. Johnson, A.J. Heck, Studying 18 MDa virus assemblies with native mass spectrometry, *Angew. Chem., Int. Ed.* 52 (2013) 4020-4023.
- [20] P.B. O'Connor, F.W. McLafferty, Oligomer characterization of 4-23 kDa polymers by electrospray Fourier transform mass spectrometry, *J. Am. Chem. Soc.* 117 (1995) 12826-12831.

- [21] R.F. Wuerker, H. Shelton, R. Langmuir, Electrodynamic containment of charged particles, *J. Appl. Phys.* 30 (1959) 342-349.
- [22] M.A. Philip, F. Gelbard, S. Arnold, An absolute method for aerosol particle mass and charge measurement, *J. Colloid Interface Sci.* 91 (1983) 507-515.
- [23] G. Hars, Z. Tass, Application of quadrupole ion trap for the accurate mass determination of submicron size charged particles, *J. Appl. Phys.* 77 (1995) 4245-4250.
- [24] S. Schlemmer, J. Illema, S. Wellert, D. Gerlich, Nondestructive high-resolution and absolute mass determination of single charged particles in a three-dimensional quadrupole trap, *J. Appl. Phys.* 90 (2001) 5410-5418.
- [25] Z. Nie, Y. Tzeng, H. Chang, C. Chiu, C. Chang, C. Chang, M. Tao, Microscopy-Based mass measurement of a single whole virus in a cylindrical ion trap, *Angew. Chem., Int. Ed.* 45 (2006) 8131-8134.
- [26] Y. Cai, W.P. Peng, S.J. Kuo, Y.T. Lee, H.C. Chang, Single-particle mass spectrometry of polystyrene microspheres and diamond nanocrystals, *Anal. Chem.* 74 (2002) 232-238.
- [27] R.D. Smith, X. Cheng, J. Brace, S. Hofstadler, G. Anderson, Trapping, detection and reaction of very large single molecular ions by mass spectrometry, *Nature* 369 (1994) 137-139.
- [28] X. Cheng, R. Bakhtiar, S. Van Orden, R.D. Smith, Charge-state shifting of individual multiply-charged ions of bovine albumin dimer and molecular weight determination using an individual-ion approach, *Anal. Chem.* 66 (1994) 2084-2087.
- [29] J.E. Bruce, X. Cheng, R. Bakhtiar, Q. Wu, S. Hofstadler, G. Anderson, R.D. Smith, Trapping, detection, and mass measurement of individual ions in a Fourier transform ion cyclotron resonance mass spectrometer, *J. Am. Chem. Soc.* 116 (1994) 7839-7847.
- [30] R. Chen, Q. Wu, D.W. Mitchell, S.A. Hofstadler, A.L. Rockwood, R.D. Smith, Direct charge number and molecular weight determination of large individual ions by electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry, *Anal. Chem.* 66 (1994) 3964-3969.
- [31] R. Chen, X. Cheng, D.W. Mitchell, S.A. Hofstadler, Q. Wu, A.L. Rockwood, M.G. Sherman, R.D. Smith, Trapping, detection, and mass determination of coliphage T4 DNA ions by electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry, *Anal. Chem.* 67 (1995) 1159-1163.
- [32] A. Makarov, E. Denisov, Dynamics of ions of intact proteins in the orbitrap mass analyzer, *J. Am. Soc. Mass Spectrom.* 20 (2009) 1486-1495.
- [33] R.J. Rose, E. Damoc, E. Denisov, A. Makarov, A.J. Heck, High-sensitivity orbitrap mass analysis of intact macromolecular assemblies, *Nat. Methods* 9 (2012) 1084-1086.
- [34] A.G. Marshall, C.L. Hendrickson, G.S. Jackson, Fourier transform ion cyclotron resonance mass spectrometry: A primer, *Mass Spectrom. Rev.* 17 (1998) 1-35.
- [35] A. Makarov, Electrostatic axially harmonic orbital trapping: A high-performance technique of mass analysis, *Anal. Chem.* 72 (2000) 1156-1162.

- [36] H. Shelton, C.D. Hendricks, R.F. Wuerker, Electrostatic acceleration of microparticles to hypervelocities, *J. Appl. Phys.* 31 (1960) 1243-1246.
- [37] C.D. Hendricks Jr., Charged droplet experiments, *J. Colloid Sci.* 17 (1962) 249-259.
- [38] P.W. Keaton, G.C. Idzorek, L.J. Rowton Sr., J.D. Seagrave, G.L. Stradling, S.D. Bergeson, M.T. Collopy, H.L. Curling Jr., D.B. McColl, J.D. Smith, A hypervelocity - microparticle - impacts laboratory with 100-km/s projectiles, *Int. J. Impact Eng.* 10 (1990) 295-308.
- [39] G.L. Stradling, G.C. Idzorek, B.P. Shafer, H.L. Curling Jr., M.T. Collopy, A.A.H. Blossom, S. Fuerstenau, Ultra-high velocity impacts: Cratering studies of microscopic impacts from 3 km/s to 30 km/s, *Int. J. Impact Eng.* 14 (1993) 719-727.
- [40] S.D. Fuerstenau, W.H. Benner, Molecular weight determination of megadalton DNA electrospray ions using charge detection time-of-flight mass spectrometry, *Rapid Commun. Mass Spectrom.* 9 (1995) 1528-1538.
- [41] S.D. Fuerstenau, W.H. Benner, J.J. Thomas, C. Brugidou, B. Bothner, G. Siuzdak, Mass spectrometry of an intact virus, *Angew. Chem., Int. Ed.* 40 (2001) 542-544.
- [42] J.T. Maze, T.C. Jones, M.F. Jarrold, Negative droplets from positive electrospray, *J. Phys. Chem. A* 110 (2006) 12607-12612.
- [43] T. Doussineau, M. Kerleroux, X. Dagany, C. Clavier, M. Barbaire, J. Maurelli, R. Antoine, P. Dugourd, Charging megadalton poly(ethylene oxide)s by electrospray ionization. A charge detection mass spectrometry study, *Rapid Commun. Mass Spectrom.* 25 (2011) 617-623.
- [44] T. Doussineau, C.Y. Bao, R. Antoine, P. Dugourd, W. Zhang, F. D'Agosto, B. Charleux, Direct molar mass determination of self-assembled amphiphilic block copolymer nanoobjects using electrospray-charge detection mass spectrometry, *ACS Macro Lett.* 1 (2012) 414-417.
- [45] N. Ouadah, T. Doussineau, T. Hamada, P. Dugourd, C. Bordes, R. Antoine, Correlation between the charge of polymer particles in solution and in the gas phase investigated by zeta-potential measurements and electrospray ionization mass spectrometry, *Langmuir* 29 (2013) 14074-14081.
- [46] T. Doussineau, A. Désert, O. Lambert, J. Taveau, M. Lansalot, P. Dugourd, E. Bourgeat-Lami, S. Ravaine, E. Duguet, R. Antoine, Charge detection mass spectrometry for the characterization of mass and surface area of composite nanoparticles, *J. Phys. Chem. C* 119 (2015) 10844-10849.
- [47] A. Viodé, X. Dagany, M. Kerleroux, P. Dugourd, T. Doussineau, L. Charles, R. Antoine, Coupling of size-exclusion chromatography with electrospray ionization charge-detection mass spectrometry for the characterization of synthetic polymers of ultra-high molar mass, *Rapid Commun. Mass Spectrom.* 30 (2016) 132-136.
- [48] M. Gamero-Castaño, Induction charge detector with multiple sensing stages, *Rev. Sci. Instrum.* 78 (2007) 043301.
- [49] J.W. Smith, E.E. Siegel, J.T. Maze, M.F. Jarrold, Image charge detection mass spectrometry: Pushing the envelope with sensitivity and accuracy, *Anal. Chem.* 83 (2011) 950-956.

- [50] B.L. Barney, R.T. Daly, D.E. Austin, A multi-stage image charge detector made from printed circuit boards, *Rev. Sci. Instrum.* 84 (2013) 114101.
- [51] R. Grix, R. Kutscher, G. Li, U. Grüner, H. Wollnik, H. Matsuda, A time-of-flight mass analyzer with high resolving power, *Rapid Commun. Mass Spectrom.* 2 (1988) 83-85.
- [52] D. Zajfman, O. Heber, L. Vejby-Christensen, I. Ben-Itzhak, M. Rappaport, R. Fishman, M. Dahan, Electrostatic bottle for long-time storage of fast ion beams, *Phys. Rev. A* 55 (1997) R1577-R1580.
- [53] S. Ring, H.B. Pedersen, O. Heber, M.L. Rappaport, P.D. Witte, K.G. Bhushan, N. Altstein, Y. Rudich, I. Sagi, D. Zajfman, Fourier transform time-of flight mass spectrometry in an electrostatic ion beam trap, *Anal. Chem.* 72 (2000) 4041-4046.
- [54] D. Zajfman, Y. Rudich, I. Sagi, D. Strasser, D.W. Savin, S. Goldberg, M. Rappaport, O. Heber, High resolution mass spectrometry using a linear electrostatic ion beam trap, *Int. J. Mass Spectrom.* 229 (2003) 55-60.
- [55] R.T. Hilger, R.E. Santini, S.A. McLuckey, Nondestructive tandem mass spectrometry using a linear quadrupole ion trap coupled to a linear electrostatic ion trap, *Anal. Chem.* 85 (2013) 5226-5232.
- [56] W.H. Benner, A gated electrostatic ion trap to repetitiously measure the charge and m/z of large electrospray ions, *Anal. Chem.* 69 (1997) 4162-4168.
- [57] T. Doussineau, C. Yu Bao, C. Clavier, X. Dagany, M. Kerleroux, R. Antoine, P. Dugourd, Infrared multiphoton dissociation tandem charge detection-mass spectrometry of single megadalton electrosprayed ions, *Rev. Sci. Instrum.* 82 (2011) 084104.
- [58] T. Doussineau, R. Antoine, M. Santacreu, P. Dugourd, Pushing the limit of infrared multiphoton dissociation to megadalton-size DNA ions, *J. Phys. Chem. Lett.* 3 (2012) 2141-2145.
- [59] R. Antoine, T. Doussineau, P. Dugourd, F. Calvo, Multiphoton dissociation of macromolecular ions at the single-molecule level, *Phys. Rev. A* 87 (2013) 013435.
- [60] E.E. Pierson, N.C. Contino, D.Z. Keifer, M.F. Jarrold, Charge detection mass spectrometry for single ions with an uncertainty in the charge measurement of 0.65 e, *J. Am. Soc. Mass Spectrom.* 26 (2015) 1213-1220.
- [61] N.C. Contino, M.F. Jarrold, Charge detection mass spectrometry for single ions with a limit of detection of 30 charges, *Int. J. Mass Spectrom.* 345-347 (2013) 153-159.
- [62] N.C. Contino, E.E. Pierson, D.Z. Keifer, M.F. Jarrold, Charge detection mass spectrometry with resolved charge states, *J. Am. Soc. Mass Spectrom.* 24 (2013) 101-108.
- [63] D.Z. Keifer, D.L. Shinholt, M.F. Jarrold, Charge detection mass spectrometry with almost perfect charge accuracy, *Anal. Chem.* 87 (2015) 10330-10337.
- [64] E.E. Pierson, D.Z. Keifer, N.C. Contino, M.F. Jarrold, Probing higher order multimers of pyruvate kinase with charge detection mass spectrometry, *Int. J. Mass Spectrom.* 337 (2013) 50-56.

- [65] D.Z. Keifer, E.E. Pierson, J.A. Hogan, G.J. Bedwell, P.E. Prevelige, M.F. Jarrold, Charge detection mass spectrometry of bacteriophage P22 procapsid distributions above 20 MDa, *Rapid Commun. Mass Spectrom.* 28 (2014) 483-488.
- [66] E.E. Pierson, D.Z. Keifer, L. Selzer, L.S. Lee, N.C. Contino, J.C.-. Wang, A. Zlotnick, M.F. Jarrold, Detection of late intermediates in virus capsid assembly by charge detection mass spectrometry, *J. Am. Chem. Soc.* 136 (2014) 3536-3541.
- [67] E.E. Pierson, D.Z. Keifer, A. Asokan, M.F. Jarrold, Resolving adeno-associated viral particle diversity with charge detection mass spectrometry, *Anal. Chem.* 88 (2016) 6718-6725.
- [68] H.T. Schmidt, H. Cederquist, J. Jensen, A. Fardi, Conetrap: A compact electrostatic ion trap, *Nucl. Instr. and Meth. in Phys. Res. B* 173 (2001) 523-527.
- [69] X.L. Chen, S.A. Jenekhe, Solubilization and encapsulation of fullerenes by amphiphilic block copolymers, *Langmuir* 15 (1999) 8007-8017.
- [70] M. Kremer, E. Pothmann, T. Roessler, J. Baker, A. Yee, H. Blanch, J.M. Prausnitz, Pore-size distributions of cationic polyacrylamide hydrogels varying in initial monomer concentration and cross-linker/monomer ratio, *Macromolecules* 27 (1994) 2965-2973.
- [71] S.F. Wong, C.K. Meng, J.B. Fenn, Multiple charging in electrospray ionization of poly(ethylene glycols), *J. Phys. Chem.* 92 (1988) 546-550.
- [72] T. Nohmi, J.B. Fenn, Electrospray mass spectrometry of poly(ethylene glycols) with molecular weights up to five million, *J. Am. Chem. Soc.* 114 (1992) 3241-3246.
- [73] E.W. Becker, K. Bier, W. Henkes, Strahlen aus kondensierten atomen und molekeln im hochvakuum, *Z. Physik* 146 (1956) 333-338.
- [74] J. Fernandez de la Mora, Electrospray ionization of large multiply charged species proceeds via Dole's charged residue mechanism, *Anal. Chim. Acta* 406 (2000) 93-104.

Figures

Fig. 1: Schematic diagrams of a) the single particle analyzer of mass (SPAM) instrument and b) the combined ion trap and detector array.

Fig. 2: Comparison of trapping performance of cone trap and electrostatic ion trap (EIT), showing a) position of the ion inside the two traps as a function of time, b) the length of time an ion is trapped relative to the maximum trapping time of 50 ms at different trapping voltages, and c) entrance radii, and d) the length of time an ion is trapped for at different entrance angles at pressures of 10^{-5} , 10^{-6} , and 10^{-7} Torr.

Fig. 3: Transient recorded for a single polystyrene nanoparticle ion that was trapped for 63 ms.

The upper transient shows the data from channel A, and the lower transient the data from channel B. The inset is an expansion of both channels between 10.6 ms and 12.75 ms.

Fig. 4: Data showing percent uncertainty in measured charge versus the length of ion trapping time for 376 PEG ions with a nominal 8 MDa mass. The inset shows the trapping time of all ions with an absolute charge uncertainty less than 10 charges, as low as 2 charges for ions trapped for approximately 100 ms.

Fig. 5: Histogram of 376 PEG ion masses making up a mass spectrum of a sample of nominal molecular weight 8 MDa.

Fig. 6: Data from a nominal 50 nm amine-modified polystyrene nanoparticle sample: a) mass spectrum measured with SPAM, b) size distribution determined from mass spectrum, c) size distribution determined from TEM images, and d) TEM image of the nanoparticle sample.

Fig. 7: Measured charge of ions from the 50 nm nanoparticle sample versus their diameter determined from the mass data obtained with SPAM. The line shows the Rayleigh limit charge for a water droplet of a given size. Relatively few ions are detected below ~400 charges, indicating the threshold for detecting an ion from a single induced signal.

Fig. 8: Data from a nominal 100 nm amine-modified polystyrene nanoparticle sample: a) mass spectrum measured by SPAM, b) size distribution determined from the mass spectrum, c) size distribution determined from TEM images, d) relative trapping rate at each particle size, e) TEM image of the nanoparticle sample.

Fig. 9: Size distribution of ions from a nominal 100 nm amine-modified polystyrene nanoparticle sample measured at different trapping voltages, a) 300 V, b) 310 V, c) 320 V, d) 330 V, e) 340 V, f) 350 V, g) 360 V, h) 370 V, i) 380 V, and j) 390 V.