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Increasing the Trapping Mass Range to $m/z = 10^9$ —A Major Step Toward High Resolution Mass Analysis of Intact RNA, DNA and Viruses

Xinyu Wang, Huijuan Chen, Jeonghoon Lee, and Peter T. A. Reilly*

Washington State University, Department of Chemistry, Fulmer 128, Pullman, WA 99164

Abstract

This work demonstrates sampling of singly-charged particles up to 200 nm in diameter at atmospheric pressure into vacuum and trapping large numbers ($>10^6$) at a point in front of the end cap electrode of a linear quadrupole ion guide/trap for on-demand injection into the acceleration region of a time-of-flight mass spectrometer in a well-collimated ion packet. This procedure was shown to yield trapping efficiencies that ranged from 4–5 percent for 10 nm diameter urea particles (~ 400 kDa) to 1 percent for 200 nm urea particles ($\sim 3 \times 10^9$ Da). Analysis of the inlet optimization procedure suggests that the inlet can be adapted to sample and trap beyond the 200 nm range. Review of the most likely places for ion loss in the sampling process suggests that the sampling and trapping efficiencies can be improved well beyond the 4–5 percent shown. Moreover, it suggests that sampling of smaller than 10 nm ions could achieve efficiencies in the 10's of percent range thereby suggesting new levels of sensitivity can be achieved for small ions (< 200 kDa). Finally, demonstration of trapping large numbers of 200 nm (3×10^9 Da) ions for on-demand ejection in well collimated temporally discrete ion packets is a prelude to resolved mass analysis in that range.

Introduction

A mass spectrometer can be thought of as a window into the mass specific universe. Anything in the mass specific universe can then be analyzed provided it can be made to fit within that window. Unfortunately, the width of window does not fit the range of the mass specific species needed to be analyzed. For example, proteins range up to roughly 200 kDa and protein complexes can range into the MDa regime while DNA and viruses can range up into the gigadalton regime (approximately 200–300 nm in size). Currently available commercial mass spectrometers provide a window that is much smaller than even the range of proteins. Consequently, most of these biological mass specific species have to be fragmented or multiply charged to be accessible to mass analysis.

Unfortunately, fragmenting and multiply charging the analyte has limitations. The spectra become more spectrally dense with increasing mass and often yield poorer resolution and or sensitivity at the same value of m/z . It is for these reasons, that we do not currently have a way to rapidly mass analyze intact really massive analytes such as RNA, DNA and viruses.

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*CORRESPONDING AUTHOR: Peter T. A. Reilly, pete.reilly@wsu.edu, 509-335-0042.

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In order to make this happen, the working range of mass spectrometers has to be dramatically extended.

The extension of the working range is not a trivial task. Many groups in academia, national labs and industry from around the world have worked on this problem since the advent of electrospray and MALDI in the late 1980's with minimal progress until now. Analysis of the problem reveals that expansion into vacuum imparts a large amount of kinetic energy to the massive analytes. The average and spread of the analyte kinetic energy distribution increases monotonically with mass and competes with and then overwhelms the motion that occurs when the mass spectrometer fields are applied. As a result, mass spectrometer resolution and sensitivity decrease with increasing mass. To significantly extend the mass spectrometer range the expansion induced kinetic energy has to be eliminated. To be precise, the massive ions have to be expanded into vacuum and trapped in a buffer gas until the residual kinetic energy is dissipated through collisions. Once this happens, the motions of the ions are then completely defined by the applied fields and resolved mass analysis can be performed.

It may be argued that massive ions have been trapped before. For example one of the first uses of the Paul trap was to trap micrometer sized aluminum particles;[1] however, this experiment was performed at atmospheric pressure. Massive ions have also been trapped in vacuum. Smith's group has trapped individual highly charged T4 DNA molecules (110 MDa).[2] Fuerstenau et al.[3] mass analyzed a plurality of intact highly charged tobacco mosaic virus particles (40.5 MDa). Peng et al.[4] acoustically desorbed highly-charged whole cells and microparticles (up to 10^{14} Da) into a 3-D ion trap. In each these cases, the charge states were unresolved or the value of the charge z is imprecisely determined. Even though Smith's group could precisely measure the mass-to-charge ratio of the single intact DNA ion that they trapped, they could only determine the charge within ten percent.[2] Consequently, they could only determine the ion mass to within ten percent. In each of these cases, the charge of the bioparticles could not be precisely determined. In fact it is so indeterminate that all of these measurements are not analytically useful. If those measurements were made on an unknown, they would have very little value.

Consequently, the charge has to be precisely known to make resolved mass analysis in this range feasible. The greater the value of the charge z , the more difficult it is to precisely determine. Conversely, if the charge is small, it is easier to precisely define; however, it is much harder to electrostatically manipulate the ions because the applied force is proportional to the charge. Large forces are needed to compensate for the mass related expansion-induced kinetic energy. We suggest that this is the reason that reducing the charge to mass analyze large ions has never been a well-trying approach.

Previously, our group demonstrated a new inlet and digitally-driven trapping system that can be used to sample singly-charged massive ions from the atmosphere and trap them in a linear quadrupole ion trap at low pressure.[5] Later, we integrated our inlet and trapping system with an orthogonal acceleration time-of-flight mass analyzer and demonstrated that resolved mass analysis of singly-charged intact proteins could be obtained [6] using digital waveform manipulation [7] to inject the trapped ions into the mass analyzer in well-collimated temporally discrete ion packets. The mass range of the prototype instrument is limited by the detector (Photonis Bipolar Detector). The manufacturer defines the detection limit at approximately $m/z = 10^6$. However, we have always maintained that the range of the inlet and trapping system was not so limited. In this work, we used Faraday detection to demonstrate for the first time that millions of singly-charged particulate ions up to 200 nm in diameter (up to $m/z = 3 \times 10^9$) can be sampled from the atmosphere, trapped in a digitally-driven linear quadrupole ion trap to be ejected on demand in well collimated ion packets into a mass analyzer with great efficiency using our technique.

Method

The experimental setup used is schematically represented in Figure 1. Polydisperse urea aerosols were produced from 1:1 methanol/water solutions with a Collison nebulizer, dried by passage through a heated tube to desolvate the wet particles and then through an ice bath cooled tube to condense the solvent. Dried polydisperse urea aerosols were then sampled into a commercial differential mobility analyzer (DMA) (Model 3085 and 3081, TSI Inc., Roadshore View, Minnesota). The DMA uses a Kr-85 source to neutralize the charge distribution on the airborne particles. The neutralization process centers the charge distribution at zero charge; however, significant portions of the distribution are singly charged with both polarities. The neutralized aerosol then passes into the DMA. The potentials of the DMA are set so that a narrow range of particle sizes of one polarity passes through the DMA to the exit. Neutral particles and charged particles outside the selected range do not pass through the DMA.

The flow rate through the DMA is measured by the pressure drop across an impactor at the front of the DMA. The output of size-selected monodisperse primarily singly-charged urea aerosol exiting the DMA is split. A small fixed flow is sampled into the mass spectrometer. The rest of the roughly 2 L/min flow is sampled into a charged aerosol detector (see the inset of Figure 1). The flow rate through the charged aerosol detector is given by the flow rate through the DMA minus the mass spectrometer inlet flow rate. The charged aerosol detector uses a biased Faraday plate to measure the current produced by the charged aerosol impacting on the Faraday plate. Knowing the current and the volume flow rate through the charged aerosol detector, the number density of the size selected singly-charged particles exiting the DMA can be determined.

The mass spectrometer inlet flow is fixed by the diameter of the flow limiting orifice and the pressure drop across it. For a 100 μm diameter orifice, that is the standard of atmospheric sampling aerodynamic lens inlet systems, the flow is roughly 100 scc/min. Our 100 μm diameter inlet also has a 100 scc/min flow rate.

When the singly-charged aerosol enters the mass spectrometer through the flow limiting orifice, it undergoes an adiabatic expansion inside a standard $\frac{3}{4}$ inch OD Quick Flange tube and then flows into a plenum chamber where it undergoes further expansions and then finally exits into the large radius quadrupole (LRQ) chamber. The pressure inside the plenum chamber is easily adjusted with the micrometer by changing the spacing between the cylinder and the final expansion orifice. The plenum chamber pressure was measured with a thermocouple gauge.

The progression of the aerosol from the inlet through the plenum chamber and out into the large radius quadrupole (LRQ) can be described as follows. The aerosol undergoes an adiabatic expansion as it passes through the flow limiting orifice into the tube with a 340 mTorr background pressure. The carrier gas, because of its relatively low inertia, quickly converts to a Laminar flow. Because the aerosol particles have much larger inertia coming out of the expansion, their velocity is greater than the linear velocity of the carrier gas in the tube. The aerosol particles rapidly lose inertia through collisions with the carrier gas until their forward velocity matches the linear velocity of the carrier gas. When this happens, the motion of the particles in the tube and then the plenum chamber is essentially defined by the gross motion of the carrier gas. The flow of the carrier gas is defined by the shape of the chamber. Therefore, the particles exit the entrance tube and follow the bend of the chamber walls and move toward the exit. The spacing between the cylinder and the final expansion orifice plate creates an expansion slit with a lower pressure at the center of the cylinder. The carrier gas and airborne particles expand through this slit toward the center of the cylinder

(see Figure 1). Because the slit is circularly symmetric, the expansion propagates inwardly and meets itself at the center of the cylinder where it then must expand again in an overall perpendicular direction into the LRQ. The expansion from the center of the cylinder is dispersive. To increase the probability of capture by the quadrupole, the radius of the quadrupole was increased to capture more of the dispersive particle injection.

The pressure in the LRQ chamber is defined by the diameter of the flow limiting orifice and the pumping speed of the LQR chamber. A 100- μm diameter inlet orifice and a 250 L/s turbo pump on the LRQ chamber yield a pressure of 4–5 mTorr. The LQR pressure is independent of the pressure in the plenum chamber (the rate of input has to equal the output). The particles exiting the final expansion orifice are stopped inside the length of our LRQ by collisions with the 4–5 mTorr buffer gas. This permits the charged particles to be captured by the imposed quadrupole field and settle toward the central axis of the LRQ before the charged particles reach the end of the LRQ. To keep the charged particles continuously moving toward the exit after their expansion induced kinetic energy has been abated by buffer gas collisions, we employ slanted wires between the quadrupole rods.[8] Biasing the four wires with the same potential creates a roughly linear field along the quadrupole axis that keeps the charged particles moving slowly along the axis toward the exit end cap electrode.[7]

To facilitate the conversion of urea particle size into molecular weight, we have created a log-log plot of the urea particle size versus the particle mass in Daltons in Figure 2. It shows that a 200-nm diameter urea particle has a molecular mass of $\sim 3.0 \times 10^9$ Da. The enormous range of particle masses ($m/z \sim 10^9$) that we wish to cover cannot be accomplished without the use of digital waveform technology. The frequency of the trapping field has to be changed for each size range covered. A single frequency that is the norm for most multipole devices will not suffice.

Moreover, the use of digital waveforms has distinct advantages. We have shown that the duty cycle of the applied waveforms can be manipulated to trap ions or eject in a well collimated plug with a controlled kinetic energy distribution.[7] We have used this capture and ejection process to produce high resolution time-of-flight mass spectra in the ultra high mass range ($m/z > 20,000$) for the entire range of intact singly charged proteins.[6] In the configuration used in this work, the LRQ waveforms are set to eject the charged particles continuously. Figure 3 depicts the axial potentials experienced by the ions as they move from the plenum chamber through the LRQ to be trapped at the end of the SRQ and then ejected on demand into the TOF. In Figure 3, the DC quadrupole axis potentials are depicted in green (lines A and C). They are offset from ground potential to compensate for the axial ejection field imposed by changing the duty cycle of quadrupole waveforms. For example, if the duty cycle is set so that all four rods have the same positive potential for 20 % of the waveform cycle and the other 80 % yields quadrupolar potentials then the duty cycle applied axial potential is 20 % of the positive AC potential applied to the rods. The DC quadrupole axis potential (A) has to be lowered by more than the duty cycle contribution to the axial potential. As a result, the charged particles experience a small net potential drop (depicted by arrow 1 in Figure 3) in passing from the plenum chamber at ground potential into the LRQ. The axial potential that the ions experience in the LRQ is depicted as line B in Figure 3. It is defined as the sum of the DC axial potential and the duty cycle applied axial potential. The charged particles entering LRQ slow by buffer gas collisions while being caught in the quadrupole field to relax on to the quadrupole axis. Once the expansion induced forward motion has been abated by buffer gas collisions, the ions are moved by a small field imposed on the quadrupole axis by slanted wire electrodes[8] in a controlled motion continuously into the small radius quadrupole (SRQ).[6, 7]

The SRQ waveforms are set to trap the charged particles by setting the duty cycle so that for a percentage of the waveform cycle all four of the rods are at the same negative potential. The DC axis potential of the SRQ depicted by line C in Figure 3 is shifted to control the potential change during ejection. The axial potential experienced by the ions during trapping is depicted by line D in Figure 3. Line D is the sum of the DC axis potential and the duty cycle applied trapping potential. Line D is lower than the DC axis potential because the waveforms have been set to trap. The combination of the applied trapping waveforms and the central axis potential created by slanted wire electrode cause the charged particles to move from the LRQ to the SRQ (depicted by arrow 2 in Figure 3) and then migrate toward the exit end cap electrode and collect where the force from the central axis potential and the force created by the trapping waveform balance just in front of the exit end cap electrode.[7] The pressure in the SRQ is roughly the same as the LRQ (4–5 mTorr). Ions that are caught in the SRQ trap quickly settle from buffer gas collisions. The trapped ions are then ejected on demand by switching the duty cycle of the waveforms applied to the SRQ from trapping to ejection. This change in the duty cycle jumps the axial potential that the ions experience in the SRQ (depicted as arrow 3 in Figure 3) from line D to line E. The ejection waveforms push the ions out of the quadrupole in a well-collimated plug (depicted by arrow 4 in Figure 3) and where they pass through an Einsel lens system to adjust the collimation. The ions are then spatially filtered and then pass into the acceleration region of the TOF to impact on a Faraday plate for detection.

Results

The pressure in the plenum chamber was set to 340 mTorr. This condition was used for all the measurements. Only the quadrupole frequencies were changed. Each particle size/mass was trapped at a Mathieu parameter value of $q_{xy} = 0.30$. [9] The particulate ions continuously entered the mass spectrometer inlet and were collected at the end of the linear trap for a user defined period before they were ejected into the Faraday plate for detection. The collection time was varied to keep the size of the response at the Faraday plate consistent because the atmospheric concentration of particles varied from day to day and with size. An example of the response at the Faraday plate from 200 nm particles is shown in Figure 4. The current amplifier was set to a gain, G , of 10^9 V/Amp. The Faraday plate signal response was converted to the number of charges, N_C , by multiplying the signal height, h , in volts by $1/2$ of the temporal width of the response at the baseline, t_B , divided by the amplifier gain times the charge of an electron in coulombs, e : $N_C = \frac{1}{2} h t_B / G e$. The 2.5 V response in Figure 4, corresponds to roughly 1×10^6 , 200-nm singly-charged urea particles ($m/z = 3 \times 10^9$ Da, see Figure 2).

The temporal width at the baseline was consistent across the entire range of particle sizes measured (more than three orders of magnitude in mass). The average baseline width was between 125 and 150 μ s. The number of charge particles, N_C , is an approximation because the integrated area under the response is assumed to be approximated by the area of an isosceles triangle. The above equation establishes the lower limit of the number of charges trapped because the ejected ions travel through spatial filters that limit the trajectories into the TOF. The exact spatial width of the ion beam out of the quadrupole has not been measured; however, given the resolution achieved in the ultra high mass range [6] and our Simion modeling results,[7] the percentage of ions occluded during the injection process into the TOF is expected to be minimal.

Figure 5 (a) reveals the number of ions trapped as a function of particle size. Our results show that large particles can be trapped in the millions. We point out that these results do not set limits to the numbers that can be trapped, the efficiency of trapping them nor the range of particles sizes that can be trapped. Our results are only defined for the current setup

of the inlet and trapping system. Be assured that the system can be setup to improve any of the above parameters. For example, the pressure in the plenum chamber could be further increased or the length of the inlet tube into the plenum chamber could be further lengthened to increase the trapping range.

In order to define the collection efficiency for the current inlet setup, the number of particulate ions passing through the inlet during the collection time had to be measured. Optical detection with a commercial condensation nuclei particle counter (CPC) was available as a method for defining the particle number density out of the DMA; however, at the charged particle number densities that were used in these experiments ($> 1 \times 10^6/\text{cm}^3$), the CPC will easily saturate and yield an inaccurate measurement. We did not want to add the further complication of diluting the aerosol by several orders of magnitude into the detection range of the CPC. For this reason we elected to use another current amplifier to measure the rate of charged particulate ions coming out of the DMA.

The charged particle number density coming out of the DMA was determined by the change in the response at the atmospheric pressure Faraday plate. The response from this detector was on the order 15 mV at a gain of 10^9 V/A. The signal was not large because the ions were not compress into a 125–150 μs pulse of ions. Instead, they were evenly spread out over time. The number density of charged particles exiting the DMA, ρ_p , was calculated from the following expression: $\rho_p = h/GeF$, where h is the amplified detector response in volts, G is the gain factor (10^9 V/Amp), e is the charge in coulombs of an electron and F is the atmospheric pressure flow rate in cm^3/s . The charged particle number density was on the order $5 \times 10^6/\text{cm}^3$ for shown measurements. A plot of the percent trapping efficiency versus particle size is shown in Figure 5(b). Remarkably, the trapping efficiency only changes by a factor of 4 over three orders of magnitude change in the particle mass. We also point out that these sampling/trapping/detection efficiencies are as good as or better than any achieved in the small molecule range for commercial mass spectrometers. For example, Page et al. cited that only about one out of every 10^3 – 10^5 analyte ions generated by ESI at atmospheric pressure is actually detected using present instrument designs.[10] Trapping efficiencies are expected to increase with decreasing size because the particle stopping distance decreases with size. Transfer into vacuum of smaller ions such as intact proteins or even smaller molecules (peptides for example) could be optimized and significantly improved over and above the 4–5 % shown for 10 nm particles (420 kDa, singly charged) with proper inlet and trapping system design.

The concept of trapping ions at the end of a multipole ion guide using added electrodes to create a potential minimum along the z axis is not new.[8, 11] There is always a maximum to the number of ions that can be trapped. The filling of the trap is generally linear until the trap begins to saturate and the number of ions trapped levels out due to space charge effects. The number of ions trapped (BSA^+ , 66 kDa) versus collection time is shown in Figure 6 (a). The maximum number of ions trapped in our instrument ranged from 6 to 7 million. The filling rate of the trap was linear up to roughly 2 million ions in our initial design.

Another important quality of ion traps when they are used for analytical analysis is their leak rate. The easiest way to determine this quality is to saturate the trap and measure the number of ions ejected as a function of hold time. Figure 6 (b) reveals the detector response converted to the number of charges detected after saturating the trap with approximately 7 million BSA^+ ions then turning off the ion injections and then varying the amount of time that the ions are held before they are ejected into the TOF to be detected at the Faraday plate. The plot in Figure 6 (b) reveals a linear leak rate until approximately 2 million ions are reached. The leak rate then drops to zero at approximately 1 million ions. The leak rate is slow. It takes more than an hour for the number of ions trap to drop from 7 to 1 million

where the leak rate is negligible. We further point out that the spectra shown in reference [6] were observed with approximately 1 million ions injected into the TOF. The signal-to-noise levels in the reference [6] spectra were on the order of 1000 to 1. The plot in Figure 6 (b) suggests that very small concentrations can be collected over long periods to observe well-resolved spectra with excellent signal to noise.

Discussion

A. Trapping Mass Range

The mass range of commercially available mass spectrometers is fundamentally limited by the expansion into vacuum. The expansion-induced kinetic energy and just as importantly the spread of kinetic energy increase with increasing mass. These energies can become enormous as the ion size/mass increases over the range of mass specific species. Our inlet and trapping system were designed on the premise of using aerodynamics to limit the effect of expansion into vacuum.[5] We step the expansion into vacuum. After each expansion step, we use collisions with a buffer gas to “stop” the expansion-induced motion of the ions so that their motion is defined by the carrier gas movement or the applied fields. In our design, the ions undergo three separate expansions, the first at the flow limiting orifice, the second at the slit created by the spacing between the cylinder and the end plate and the final expansion normal to the expansion through the slit into the quadrupole where the kinetic energy from the final expansion is eliminated by collisions with the buffer gas inside the quadrupole before the ions reach the end of the guide.[5] The mass limit of the inlet/trapping system is defined by the stopping distance[12] of the ions after the expansion through the flow limiting inlet orifice. The distance between the flow limiting orifice and the entrance to the plenum chamber was approximately 25 cm. The stopping distance of 200-nm diameter particles (density = 1.32 g/cm^3) assuming an initial velocity of 500 m/s out of the expansion is approximately 15 cm at 340 mTorr.[12–14] Therefore, 200 nm particles will entrain in the laminar before the flow enters the plenum chamber. Larger particles will not and so the trapping efficiency will rapidly decrease beyond 200 nm for our experimental conditions. Larger particles, however, can be efficiently sampled and trapped merely by increasing the plenum chamber pressure. This is accomplished by decreasing the distance between the cylinder and the orifice plate with the micrometer or by increasing the distance between the flow limiting orifice and the plenum chamber. Alternatively, an extra step in the expansion could conceivably be added. The message here is that we have demonstrated that 200 nm diameter singly-charged particles can be efficiently sampled and trapped, but that is NOT the size limit of the method and technique. It can easily be extended well beyond 200 nm.

The ability to stop the particles inside the linear trap is not an issue. The pressure in the large radius linear ion trap chamber is operationally approximately 5 mTorr. This pressure did not change during the experiments. The fact that the pressure required to stop such massive singly-charged particles is only 5 mTorr is a testament to the ability of our inlet design for reducing the kinetic energy of such massive ions. However, should it ever become necessary to increase the pressure in the trapping chamber, there is a gate valve in front of the turbo pump that can be used to throttle the chamber pressure. Increasing the pressure in the first quadrupole is a standard method for reducing the kinetic energy of the ions. Our methodology has not needed it; however, it is available to increase the range.

There is no upper range limit for electrostatically trapping the ions provided their forward motion is stopped within the trap. The range of m/z values trapped is easily shifted to any value merely by changing the frequency of the digitally produced waveforms.[15] This ability to digitally produce the waveforms and adjust the trapping frequency provides an essentially unlimited mass range. Given the nature of our inlet and trapping system,

virtually any mass specific species can be sampled and trapped with our inlet system. There is now essentially no mass limit to atmospheric sampling and trapping.

B. Sampling and Trapping Efficiency

The sampling and trapping efficiency observed is quite good. In fact it is comparable and better than that exhibited by most commercial mass spectrometers in the low mass range. [10] Our improvement in transmission efficiency results from the separation of the carrier gas from the ions inside of the first trap/guide. The entire throughput of the flow limiting inlet orifice is injected into the first quadrupole. No differential pumping is used. In our case, the expansion is stepped without differential pumping so that the ions are injected into the quadrupole with minimal kinetic energy yielding better trapping by the quadrupole field. The ions quickly lose any excess kinetic energy through buffer gas collisions and settle near the central axis. Because the ions are immediately caught and cooled as they are injected into the large radius quadrupole with the entirety of the carrier gas load, separation of the ions from the carrier gas is extremely efficient.

However, the decrease in sampling and trapping efficiency clearly shown in Figure 5 (b) reveals that there are opportunities for ion loss within our system. Ion loss in our system results from interaction of the ions with metal surfaces and misinjection into the quadrupole. Misinjection into the quadrupole results when the ion's kinetic energy and trajectory keep it from getting caught and redirected by the quadrupole's trapping field. Misinjection is more likely to occur at smaller ion sizes/masses; primarily because they move faster and have more disperse trajectories. Given the decrease in the sampling and trapping efficiency exhibited in Figure 5 (b), misinjection is not likely a significant source of ion loss.

The major source of ion loss comes from interaction of the particulate ions with the walls. This primarily occurs during the first expansion before the particulate ions have entrained into the laminar flow. Larger ions have longer stopping distances and therefore take longer to entrain in the laminar flow. Expansion from the flow limiting orifice is dispersive; therefore, larger particles have more opportunity to interact with the walls. Adiabatic expansion against a 340 mTorr pressure yields a stopping distance near 1 cm for 10 nm particles. The adiabatic expansion from the 100 μm flow limiting orifice occurs into a $\frac{3}{4}$ inch OD Quick Flange pipe with an inner radius of approximately 8 mm and then into the plenum chamber. This provides significant opportunity for even 10 nm particles to interact with the pipe wall and lose their charge. Larger particles will have a greater probability of losing their charge in the pipe. The trend exhibited in Figure 5 (b) fits this explanation.

The sampling and trapping efficiency can easily be improved by moving the walls away from the first expansion specifically by increasing the diameter of the pipe. One might ask, why have a pipe? A valve was needed to close off the flow from the orifice to reduce the load on turbo pump when it is not in use and to permit rapid changing of the inlet orifice in case it clogs. Larger pipes and valves can be used to increase the transmission of the larger ions. The smaller ions—anything below 10 nm including most intact proteins and many complexes—should have correspondingly higher transmission efficiencies.

The other place where interaction with the walls may occur is between the cylinder and the orifice plate. Interaction in this zone does not contribute significantly to ion loss primarily because the ions are not in this zone for a significant amount of time relative to the amount of time required for them to diffuse to the wall and lose the charge. When particles/ions are entrained in a laminar flow, their gross motion is defined by the flow of the carrier and their fine motion within the gas is defined by diffusion. The diffusion time of even 10 nm particles at these pressures over the distance between the cylinder and orifice plate relative to the transit time through the slit created by the spacing between the cylinder and the orifice

plate make the probability of charge loss there negligible.[16] Calculation of the diffusion time relative to the transit time through the entrance pipe and then through the plenum chamber reveal that charge loss at the walls once the ions are entrained in the laminar flow is also negligible.

Our analysis of the charge loss process during transit through the inlet and during capture by the linear ion trap suggest that the sampling and trapping efficiency can be improved over and above the results shown in Figure 5 (b). It also shows that efficiencies for the small particles (< 10 nm) could be significantly better than the efficiencies we measured between 10 and 200 nm. The trend in Figure 5 (b) suggests that those efficiencies could extrapolate to the 10's of percent range with unit transmission efficiency approachable with proper engineering. This work suggests that new levels of sensitivity can be achieved for mass spectrometry.

C. The Need for Quadrupole/Multipole z-Axis Potentials

Another innovation of our inlet design is the recognition of the need for the creation of a z-axis potential. Stopping ions in the middle of a multipole guide/trap requires that they diffuse out. The diffusion process can take seconds, minutes or even hours depending on the size of the ions and the pressure. This is detrimental to the ability to detect the ions in significant quantity and has long been recognized as an issue in the use of ion guides.[8] Diffusion time becomes more of an issue as the size on the ion increases. The addition of the slanted wire electrodes[8], segmented rods[17] or similar devices to create a field along the z-axis is required to keep the ions moving after their expansion induced forward motion has been stopped. If such a device is not included sensitivity becomes an extreme issue.

D. Mass Analysis into the $m/z = 10^9$ Range

Our purpose for creating this inlet and trapping system has been to increase the working range of mass spectrometers to cover the entire range of mass specific species. It has always been our contention that anything can be mass analyzed with good resolution provided that it can be trapped. We have already shown that ions in the less than 10 nm size range (< 200 kDa) can be measured with excellent resolution by time-of-flight mass analysis.[6] The ion injection temporal profile of the 10 nm ions in that publication is similar to the injection profiles of the urea particles observed in this publication (see Figure 4). This suggests that 200 nm sized particles can be measured by our mass analyzer with the same facility and resolution shown in our previous publication provided the ions can be detected. TOF detectors that are insensitive to mass exist.[18–22] These detectors usually have limited active detection area (less sensitivity) or their rise/fall time is less than desirable. The issue of rise and fall time is not really that much of a problem given that the flight times of such large ions are in the millisecond range. Detector response times even in the μ s range could then yield effective resolution. Consequently, well resolved TOF-MS analysis can be accomplished in this range.

An alternative to the TOF mass analyzer is the ion trap. When operated digitally, ion traps do not have a mass limit. The mass range of a digitally operated ion trap is changed merely by changing the trapping frequency. One of the first uses of an ion trap (circa 1959) albeit at atmospheric pressure was trapping micrometer-sized aluminum particles.[1, 23] Consequently, there is no effective mass limit to trapping ions. Moreover, ion traps do not require detectors with rapid (ns) rise times. The slower response of Faraday-type detection will not impede the resolving power. In digital ion trap mass spectrometry, the ion mass to charge ratio, m/z , is proportional to the square of the trapping waveform period. Because the error in producing digital waveforms is base in part on the jitter of the clock that reads the waveforms out,[24, 25] the precision of the digital waveforms can actually increase with $m/$

z. Consequently, the resolution of digital ion traps increases as the square root of the mass in the low mass range where the precision of the waveform is limited by temporal jitter. Given the reported mass resolution at 1.5×10^3 Da is 19,000 for digital ion traps,[25] we expect the digital ion trap resolving power to improve with increasing m/z from the 19,000 benchmark until it levels to a constant that is defined by the fluctuation or ripple of the DC power supplies used to create the high voltage waveforms. While it will be interesting to see what resolving power can be ultimately obtained, the resolving power ultimately obtained in the high mass limit is not the salient point. Rather it is that resolved mass analysis into the gigadalton range is feasible and it should yield analytically useful information that can be used to directly identify and characterize species such as RNA, DNA and even viruses.

Conclusions

This work presents a new method for atmospheric sampling and trapping of massive ions. Efficient atmospheric sampling and trapping of millions of ions into the gigadalton range (10^9 Da) has been demonstrated using our new inlet and trapping technology. Increasing this range beyond the 10^9 Da should be easily achievable. Trapping efficiencies of approximately 1–5 % for singly-charged particles in the 10 to 200 nm range (400 kDa to 3 GDa) have been measured. Sampling and trapping efficiencies in the 10's of percent are expected to be achieved for particles below 10 nm. This work suggests that new levels of sensitivity can be achieved for mass spectrometry. Finally, our achievement of trapping large numbers ($>10^6$) of singly-charged particles sampled into vacuum from the atmosphere is a prelude to the achievement of resolved mass analysis into the gigadalton range. That achievement will have a significant impact on biological mass analysis.

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***Highlights**

- A method and instrumentation for sampling massive singly-charged particulate ions from the atmosphere and efficiently trapping them in vacuum has been demonstrated.
- The inlet and trapping system was shown to operate up to $m/z = 3 \times 10^9$ with a sampling/trapping/detection efficiency of 1 %.
- Trapping larger ions is possible.
- Trapping millions of ions by this method is a prelude to mass analysis in this range.

Figure 1.

Instrumental setup for trapping and detection of singly-charged nanoparticles by Faraday detection. The left inset reveals the placement of the slanted wire electrodes that create a field along the axes of the quadrupoles. The right inset reveals the instrumental setup for determining the charged particle number density before the inlet.

Figure 2.
Log-log plot of urea particle diameter versus particle mass in Da.

Figure 3.

Depiction of the axial potentials experienced by the ions during transport from the plenum chamber into the TOF. The green lines depict the DC axial potentials of the quadrupoles. The black lines depict the net axial potentials that the ions experience defined by the sum of the DC axial and the duty cycle imposed potentials. The arrows depict the potential transitions that the ions experience during transport.

Figure 4. The amplified response ($h=2.5$ V) of the Faraday detector (gain= 10^9 V/Amp) from collection of greater than 1×10^6 200 nm singly-charged ions in the linear trap followed by on-demand ejection into the TOF.

Figure 5.

(a) Number of particles trapped versus particle diameter. The collection duration was varied to keep the detector response in the same range. (b) The particle trapping efficiency versus particle size.

Figure 6.

(a) The number of singly-charged bovine serum albumin ions ($m/z = 66$ kDa) trapped versus collection time in seconds. (b) The number of ions detected after saturated injection versus the amount of time the ions are held in the trap.