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Trapping Mode Dipolar DC Collisional Activation in the RF-Only Ion Guide of a Linear Ion Trap/Time-of-Flight Instrument for Gaseous Bio-Ion Declustering

Ian K. Webb¹, Yang Gao¹, Frank A. Londry², and Scott A. McLuckey^{1,*}

¹Department of Chemistry Purdue University West Lafayette, Indiana, USA, 47907-2084

²AB Sciex 71 Four Valley Drive Concord, Ontario, Canada L4K 4V8

Abstract

The application of dipolar DC (DDC) to the RF-only ion guide (Q0) of a hybrid quadrupole/time-of-flight (QqTOF) mass spectrometer for collision-induced declustering of large bio-ions is described. As a broadband technique, ion trap DDC collision activation (CA) is employed to decluster ions simultaneously over a relatively broad mass-to-charge range. Declustering DDC CA can yield significantly narrower peaks relative to those observed in the absence of declustering methods, depending upon the extent of non-covalent adduction associated with the ions, and can also be used in conjunction with other methods, such as nozzle-skimmer collisional activation. The key experimental variables in the DDC experiment are the dipolar DC voltage (V_{DDC}), V_{RF} , and the time over which V_{DDC} is applied. The V_{DDC}/V_{RF} ratio is key to the extent to which ion temperatures are elevated and also influences the upper mass-to-charge limit for ion storage. The V_{DDC}/V_{RF} ratio affects ion temperatures and upper m/z limit in opposing directions. That is, as the ratio increases, ion temperature increases whereas the upper m/z storage limit decreases. However, for a given V_{DDC}/V_{RF} ratio, the upper m/z storage limit can be increased by increasing V_{RF} , at the expense of the lower m/z limit for ion storage. The key value of the approach is that it affords a relatively precise degree of control over ion temperatures as well as the time over which they are elevated to the higher temperature. The utility of the method is illustrated by the application of ion trap DDC CA in Q0 to oligonucleotide, protein, and multimeric protein complex analyte ions.

INTRODUCTION

The advent of electrospray ionization¹ has enabled the application of mass spectrometry to the analysis of biopolymers, such as proteins,² and their non-covalent complexes.³ The ability to generate, transmit, and measure such ions as well as the ability to conduct tandem mass spectrometry experiments on them has enabled mass spectrometry to provide insights into structural biology.^{4,5,6} A variety of solution and instrumental conditions must be met, however, for the successful generation and observation of large covalent and non-covalent complex ions via ESI. Obviously, solution conditions amenable to complex formation must be in place, which usually involves the avoidance of solvents that give rise to denaturation and the inclusion of buffers to maintain an appropriate pH range. Once ions and charged droplets are generated by electrospray, they must be sampled and transmitted by a vacuum/atmosphere interface, such as the nozzle-skimmer⁷ or heated capillary interfaces,⁸ and admitted into the vacuum system of the mass spectrometer. In the course of the disintegration and desolvation of charged droplets and in the supersonic expansion upon

*Phone: (765) 494-5270 Fax: (765) 494-0239 mcluckey@purdue.edu.

sampling into the vacuum system,⁹ there are many opportunities for the generation of cluster ions composed of solvent and salts.^{10,11} Of particular relevance for many non-covalent complexes, the condensation of salts onto the ions upon droplet desolvation can be particularly problematic for mass determination.¹²

A variety of measures have been developed to mitigate the effects of non-specific adduction of counter-ions and polar neutral molecules to large bio-ions and their complexes. Aside from the use of clean solvents and volatile buffers, these include, for example, the use of nano-electrospray,^{13,14,15} which generates smaller droplets than conventional ESI and thereby avoids a degree of non-volatile salt concentration that occurs upon droplet desolvation,¹⁶ and various approaches to drive off relatively weakly-bound adducts. Adding a countercurrent flow of dry nitrogen (sometimes heated) was among the first methods developed for solvent removal and minimization of clustering with polar species in the gas phase.¹⁷ Another method was the inclusion of an in-source heated sampling capillary to transfer thermal energy to the droplets/clusters/ions prior to mass analysis.¹⁸ Additionally, potentials can be applied between the orifice and sampling apparatus (skimmer, lenses, capillary etc.) to allow for declustering via collisional activation in the interface region.¹⁹ Declustering within the interface region can also be effected in electrodynamic transmission devices, such as the ion funnel.²⁰ For particularly high mass ions, it has been noted that the use of an electrodynamic ion transmission or trapping device operated in the presence of relatively high gas pressures at the ion source exit is important for minimizing ion losses in the subsequent transmission of ions through the instrument.^{21,22,23} The injection of ions into such devices can also promote declustering. Collisional activation methods within the vacuum system have also been described, such as the use of the sustained off-resonance irradiation approach in Fourier transform-ion cyclotron resonance mass spectrometry²⁴ and the use of a so-called 'heating ramp' (i.e., a sweep of the ring electrode ion trap RF amplitude in conjunction with the application of a relatively low resonance excitation voltage to the end-cap electrodes) in quadrupole ion traps.²⁵ Infra-red multi-photon irradiation has also been described for bio-ion declustering.^{26,27,28}

Essentially all platforms commonly used for the study of protein complexes via tandem mass spectrometry employ electrodynamic ion transmission devices operated at relatively high pressures.^{29,30} A particularly common platform is the quadrupole/time-of-flight (Q/TOF) instrument,²³ and variations thereof. We have recently modified a Q/TOF instrument that is comprised of a transmission quadrupole (Q0) that accepts ions from the atmosphere/vacuum interface, a mass resolving quadrupole (Q1), and a quadrupole collision cell (Q2) coupled with an orthogonal acceleration TOF for the application of dipolar DC potentials across a pair of quadrupole rods in both the Q0 RF-only ion guide and Q2 collision cell.³¹ Dipolar DC (DDC) has been exploited for broad-band collisional activation of ions in 2-D³² and 3-D^{33,34} ion traps. In this work, we explore the use of DDC in Q0, in conjunction with ion trapping, as a means to add flexibility in the collisional heating of relatively large protein ions and protein complexes. The approach can be used instead of, or in conjunction with, nozzle-skimmer CA in the interface and provides new variables for ion heating, such as DDC voltage magnitude and duration of application, to provide greater control over ion heating prior to the passage of ions into Q1.

EXPERIMENTAL

Materials

Bovine serum albumin (BSA), bovine serum immunoglobulin G (IgG), carbonic anhydrase from bovine erythrocytes, ammonium acetate, piperidine and imidazole were purchased from Sigma-Aldrich (St. Louis, MO). An RNA mixmer (5'-rArUrGmGmAmAmGmGfCmAfUfCmGfCfCfUmGmGmUmU-3') was provided by

Merck & Co., Inc. (Whitehouse Station, NJ). Pyruvate kinase was provided by Dr. David Clemmer's group (Indiana University, Bloomington, IN). Methanol and glacial acetic acid were purchased from Avantor, Inc. (Phillipsburg, NJ). 25 μ M protein solutions were prepared in ammonium acetate buffer, IgG was prepared in water and the RNA mixer was prepared in a 25 μ M solution of 20/80 isopropanol/water with 25 mM piperidine and 25 mM imidazole. Samples were used without further purification.

Mass Spectrometry

A modified Q/TOF was used for all experiments (QStar XL, AB Sciex, Concord, ON, CA).³⁵ The instrument is comprised of an RF-only ion guide (Q0), an RF/DC mass resolving quad (Q1) and an RF-only collision cell (Q2) before grid extraction into the orthogonal time-of-flight (TOF) for mass analysis. Q0 and Q2 have been modified for the application of a dipolar DC potential (DDC) across a pair of rods, described elsewhere.³¹ The DC offset of one rod pair in the array is electrically decoupled and with the potential of each rod applied separately from the other rod pair by Daetalyt 3.14 software provided by AB Sciex. Ions were sampled from a home-built nano-electrospray ionization (nESI) source via a nozzle-skimmer interface. Ions were trapped in Q0 by applying repulsive DC to the exit lens of Q0. Once trapped, ions are collisionally heated by applying a dipolar DC potential across a rod set in the presence of N₂ bath gas at ~8 mTorr. Ions were then released from Q0 by applying an attractive potential gradient into Q2, cooled, and extracted into the TOF (Scheme 1). Q0 is capacitively coupled to Q1, operating at a drive frequency of = 650 kHz and an amplitude of ~55% of Q1. The inscribed radius of Q0 is $r_0=4.17$ mm. The curtain plate was removed for all experiments to facilitate observation of adducted ions. Mass spectra shown are the sum of 50–200 individual mass spectra.

RESULTS AND DISCUSSION

Background for DDC declustering

The key characteristics of DDC as a means for ion acceleration in a linear quadrupole ion trap have been described by Tolmachev et al.³² A very brief summary of key points regarding the use of DDC for broad-band ion heating is given here. The main objective is to use the dipolar electric field to displace the ions from the center of the ion trap to new equilibrium positions in a region of higher RF electric field, which results in an increase in ion velocity due to RF-heating. For polyatomic ions, the increase in ion velocity results in an increase in the ion internal energy distribution via collisions with the ambient gas. The extent to which an ion of a given mass-to-charge ratio is activated is determined by the point at which the ion displacement from the DDC electric field is counter-balanced by the trapping electric field. The latter is estimated from the negative gradient of the effective pseudopotential, V_{eff} , i.e.

$$V_{\text{eff}}(x, y) = D \left(\frac{x^2 + y^2}{r_0^2} \right) \quad (1)$$

where x and y are displacements from the center of the rod array, r_0 is the inscribed radius of the rod set, and D is the well-known pseudopotential well-depth³⁶, i.e.

$$D = \frac{qV_{\text{RF}}}{4} \quad (2)$$

V_{RF} is the zero-to-peak RF amplitude and the q parameter is given by

$$q = \frac{4zeV_{RF}}{m\Omega^2 r_0^2} \quad (3)$$

where z is the ion unit charge, m is the ion mass, and Ω is the RF frequency in rad/s. The electric field, E_{DDC} , due to the DDC voltage, V_{DDC} , for round rods in a quadrupole array has been estimated to be:³⁷

$$E_{DDC} = \frac{(0.8) V_{DDC}}{2r_0} \quad (4)$$

(Note that this differs from the original approximation provided by Tolmachev by the factor 0.8. The estimate provided by Tolmachev et al. was based on the electric field between two flat plates.) Tolmachev et al. described an “effective temperature” approach to estimate the increase in polyatomic ion temperatures subjected to DDC. The change in temperature, T_K , due to the DDC voltage is given by:

$$\Delta T_K = \frac{(0.64) m_g \Omega^2 r_0^2}{24k} \left(\frac{V_{DDC}}{V_{RF}} \right)^2 \quad (5)$$

where k is the Boltzmann constant. Relation (5) is derived under the assumptions of purely quadrupolar fields, the mass of the ion to be much greater than that of the background gas, m_g , and that changes in internal temperatures reflect increases in micro-motion velocity. (Note that the factor 0.64 differs from the original Tolmachev treatment due to the approximation in Relation (4).) T_K is directly dependent on the square of V_{DDC}/V_{RF} ratio. The lack of mass-to-charge (m/z) dependence on temperature change makes DDC CA a more-or-less broadband activation technique provided that the ions are not lost by scattering or collisions with the rods.³² There is, however, an upper m/z limit that is ultimately determined by the point at which the equilibrium displacement, r_e , reaches r_0 (i.e., $r_e/r_0 = 1$), which is m/z dependent. Using Relation (4) for E_{DDC} , the equilibrium displacement is given by:

$$r_e = \frac{(0.8) V_{DDC}}{qV_{RF}} r_0 \quad (6)$$

which leads to a value for $(m/z)_{\max}$ of

$$(m/z)_{\max} = \frac{4eV_{RF}^2}{(0.8) \Omega^2 r_0^2 V_{DDC}} \quad (7)$$

(Note that the factor of 0.8 in the denominator of Relation (7) comes from Relation (4).) The minimum m/z for a DDC experiment is usual low mass cut-off for ion storage, as determined by the point at which ions reach a q -value of 0.908, i.e.:

$$(m/z)_{\min} = \frac{4eV_{RF}}{(0.908) \Omega^2 r_0^2} \quad (8)$$

Relation (7) defines a sharp high mass cut-off. However, this relationship simply determines the point at which the average equilibrium displacement reaches a rod surface and does not take into account the finite size of the ion population, the position spread due to the secular motion, the appreciable position spread due to micro-motion, and losses due to scattering. These effects are expected to lead to a decrease in trapping efficiency as r_e increases from zero and approaches r_0 . As a result of the fact that ion storage lifetimes are expected to be

dependent upon r_e , particularly as r_e becomes a substantial fraction of r_0 , the practical useful m/z range over which DDC can be used is expected to be dependent upon the DDC time over the range of r_e values where there is a roll-off in trapping efficiency.

Single Chain Biopolymer Declustering

In Figure 1, ions derived from carbonic anhydrase (~29 kDa) illustrate the effect of Q0 DDC on species with moderate adduction. In Figure 1a, which reflects storage of ions in Q0 for 100 ms after the accumulation period, only three carbonic anhydrase charge states are prominent. (The prominent charge state labeled 5^+ arises from bovine ubiquitin contamination in the sample.) The 12^+ , 11^+ , and 10^+ charge state peaks are relatively broad and show tailing on the high mass side indicative of adduction. The spectrum is considerably declustered by the application of DDC CA at $34 V_{DDC}$ (Figure 1b) for 100 ms. ($34 V_{DDC}$ refers to the simultaneous application of +17 V and -17 V to opposing rods in the Q0 array.) The nominal change in internal temperature of the ions, as predicted by Relation (5), is nominally increased by 335 K via application of $34 V_{DDC}$ with $300 V_{RF}$. The increase in ion temperature is effective in removing relatively weakly bound adducts, such as residual polar molecules and salts. Ion heating is not expected to remove metal counterions bound to negative charge sites, such as carboxylate groups. Not only are the 10 – 12^+ charge states peaks narrower in Figure 1b, charge states 13 – 19^+ are clearly distinguishable from the baseline. The 9^+ peak is also observed due to the four-fold increase in signal-to-noise ratio. The results of this experiment demonstrate declustering over a relatively wide m/z range. Discrimination at m/z values in excess of roughly m/z 3500 is also apparent in Figure 1b, despite the fact that $(m/z)_{max}$ from Relation (7) is roughly m/z 4400. We note, however, that $(m/z)_{max}$ can be increased for a given T_K by increasing V_{RF} while maintaining a constant V_{DDC}/V_{RF} ratio (compare Relations (5) and (7)) at the expense of $(m/z)_{min}$ (Relation (8)).

Anions derived from negative nESI of an RNA mixmer (sequence: 5'-rArUrGmGmAmAmGmGfCmAfUfCmGfCfCfUmGmGmUmU-3', 6.9 kDa) were examined with and without Q0 DDC (Figure 2). In the absence of a Q0 trapping period (i.e., ions were transmitted through Q0), charge states from 9^- to 4^- were observed along with charge states of a few sequence ions (e.g., those of y_{18} and y_{19}) (Figure 2a). The sequence ions were likely present in solution with minimal contribution from beam-type CID, as only a 4 V gradient was used to pass the anions from Q0 to Q2. (The abundances of the ions were not sensitive to this voltage when it was altered by several volts in either direction.) Throughout the mass spectrum are distributions of clusters of piperidine and imidazole, electrospray additives used to sequester metal cations in solution.³⁸ The ions in Figure 2b were trapped in Q0 for 200 ms in the absence of DDC. Substantial declustering is observed without applying DDC, as is often seen for particularly weakly-bound adducts. The resulting mass spectrum also shows the y_{18}^{3-} sequence ion, which was likely buried in the chemical noise of Figure 2a. $10 V_{DDC}$ was applied in Q0 to allow for additional declustering from DDC CA (Figure 2c). With the Q0 $V_{RF} = 263$ V, q for the 4^- charge state = 0.2, yielding a calculated increase in ion temperature of 38 K. This modest increase in energy resulted in less clustering and increased overall signal levels, with a particularly dramatic improvement in the signals for the y_{18}^{3-} and the 3^- charge state peaks. The signal-to-noise ratio of the 3^- peak was increased from essentially 0 (Figure 2a) to 2 (Figure 2b) and to over 34 (Figure 2c). Figures 1 and 2 together illustrate the wide range of temperature changes that can nominally be effected by the Q0 DDC experiment applied to single chain biopolymer anions and cations.

Effects of V_{DDC} , V_{RF} , and DDC Time

Figure 3 illustrates Q0 DDC applied to cations derived from positive ion nESI of the pyruvate kinase tetramer, a roughly 232 kDa protein complex. Figure 3a shows the result

obtained from trapping the ions in Q0 for 5s in the absence of any DDC before TOF mass analysis. Several of the charge states of the tetramer can be distinguished with the last peak being quite broad with a long high m/z tail. The application of 200 V_{DDC} for 10 ms during a 5s storage period reveals that the large peak is in fact an envelope of unresolved charge states (Figure 3b). Six charge states appear in the high mass portion of the spectrum. The internal temperatures of the tetramer cations were nominally raised by 489 K (1460 V_{RF} and $q = .26$). CID of protein complexes produces charge-asymmetric fragments; from tetramers, highly charged monomers and low charge trimers typically result.³⁹ There is no direct evidence of disruption of the complex in this case as neither monomer nor trimer peaks are observed as a result of the DDC voltage.

For a given RF frequency and background gas, the experimental variables in a DDC CA experiment are V_{DDC}, V_{RF}, and time. V_{DDC} and V_{RF} are key factors in determining T_K and $(m/z)_{\max}$ via Relations (5) and (7), respectively. V_{RF} is also a key factor in determining $(m/z)_{\min}$ (Relation (8)). The time over which V_{DDC} is applied determines the integration time for the processes that occur at the elevated temperatures associated with the RF-heating. The effects of these variables are illustrated here with ions derived from pyruvate kinase. The effect of Q0 V_{RF} at fixed time (100 ms) and V_{DDC} (247 V) is shown in Figure 4. Figure 4a–c shows the results obtained with Q0 operated at 1607, 1460, and 1315 V_{RF}, respectively. Figure 3a shows results in the absence of V_{DDC} for 1460 V_{RF}. Despite the much longer storage time in Figure 3a (5 s), the 100 ms V_{DDC} of Figure 4b clearly shows significant declustering, as evident from the resolved charge states on the high mass side of the 31⁺ charge state. These charge states were buried beneath the tail of the large unresolved peak in Figure 3a. Figure 4a (1607 V_{RF}) reflects a nominal T_K of 520 K whereas Figure 4b (1460 V_{RF}) reflects a nominal T_K of 630 K. The additional heating at 1460 V_{RF} caused a noticeable increase in declustering for all charge states, increasing signal-to-noise ratios by roughly a factor of 2. The use of 1315 V_{RF} in Figure 4c results in a calculated value of $(m/z)_{\max}$ of roughly m/z 11,800. However, essentially all of the ions are lost over the course of 100 ms under these conditions, which reflects the loss of ion storage efficiency as r_e approaches r_0 . The calculated r_e values for the 31⁺ charge state for 1460 V_{RF} and 1315 V_{RF} at a V_{DDC} are 2.4 and 2.7 mm, respectively, which suggests a relatively rapid drop-off in storage efficiency within this range.

Figure 5 demonstrates the effect of V_{DDC} with constant V_{RF} (1315 V) and heating time (100 ms). Increasing V_{DDC} is predicted to increase effective temperatures by the square of the V_{DDC}. Using 167 V_{DDC} (nominal $T_K = 419$ K) yields a modest degree of declustering while raising the DDC voltage to 205 V_{DDC} (nominal $T_K = 633$ K) (Figure 5b) significantly narrows the peaks in the charge state envelope relative to those in Figure 5a and increases signal-to-noise ratios by as much as a factor of 25, revealing several lower charge peaks that were in the chemical noise of the previous spectrum. However, when the DDC voltage is increased to 239 V_{DDC}, the lower charge peaks again disappear into chemical noise, reducing signal-to-noise by almost 200-fold (Figure 5c). In this case, the ion displacement is becoming sufficiently high to compromise trapping efficiency. A further increase in V_{DDC} results in an even greater loss of signal (see Figure 4c). Figures 4 and 5 indicate similar qualitative trends are observed for lowering the RF amplitude and increasing DDC voltage, as is suggested by Relation (5).

DDC CA is a form of slow heating with analogies to conventional resonance excitation ion trap CA, boundary-activated dissociation in ion traps, continuous-wave infra-red multiphoton dissociation, sustained off-resonance irradiation in ion cyclotron resonance, etc.⁴⁰ These methods tend to give rise to a steady-state internal energy distribution and, for most experiments in mass spectrometry, conditions are used to yield ion lifetimes over the range of milliseconds to seconds. Increasing the activation time allows for a greater integration of

dissociation products. In the case of DDC CA, all ions are elevated nominally to the same temperature such that stepwise reactions can be facilitated by use of long activation times. This effect has already been reported for DDC tandem mass spectrometry experiments.^{31,32} The sensitivity of spectra of extensively adducted ions to the DDC CA time is dependent upon T_K and the energy-dependent lifetimes of the various cluster/adduct species present in the ion population. Relatively weakly-bound species, for example, can be expected to fragment relatively quickly whereas strongly bound adducts, such as metal ions associated with carboxylate groups in a protein, are expected to be relatively insensitive to activation time under conditions in which covalent bonds remain intact. Ions from the pyruvate kinase tetramer are used here to illustrate the effect of activation time for a large protein complex (Figure 6). The spectra of Figures 6a–d reflect DDC activation times of 10, 50, 200, and 1600 ms, respectively, using 1607 V_{RF} and 212.5 V_{DDC} (nominal $T_K = 456$ K). The spectra show an evolution that reflects a gradual increase in declustering with time up until at least 200 ms. At the longest time (1600 ms), further declustering of the lowest charge states is apparent but there is also a loss of signal due to the finite trapping efficiency in Q0. We conducted a comparable set of experiments with a 10 ms DDC period with various subsequent storage times in Q0 (i.e., 10 ms DDC followed by ion storage for 0 ms, 40 ms, 190 ms, and 1590 ms, respectively), which showed essentially no change in the spectra as the ion storage time in the absence of DDC was extended. This result indicates that the evolution of the spectra in Figure 6 results from DDC. Some changes in the relative abundances of the charges states are noted as the DDC activation time is extended (compare Figure 6(a) with Figure 6(c), for example). A possible rationale for the changes observed in Figure 6 is that some of the declustering involves the loss of one or more small cations, which accounts for the growth in the relative abundances of the lower charge states in Figure 6(c). The storage efficiencies for higher mass-to-charge ratio ions, however, are likely to be lower than those for the more highly charged ions (i.e., the lower mass-to-charge ratio ions). The discrimination at high m/z ratio is expected to be greatest at the longest storage time (Figure 6(d)), where the effects of ion evaporation from the trap are most pronounced.

Nozzle-skimmer and DDC Declustering

The use of relatively high voltage gradients in ESI interfaces (e.g., nozzle-skimmer CA) for declustering is commonplace and simple to perform. The time-scale of the ion heating, however, is not readily controlled, the ion heating is not expected to be uniform across the various charge states, and there are trade-offs between ion transmission efficiency and voltage gradient. The ability to perform a DDC experiment in Q0 provides an independent option for ion heating with relatively precise control over time and temperature instead of or in addition to ion acceleration in the interface. Figure 7 illustrates results using nozzle-skimmer CA, DDC, and a combination of the two approaches with the protein bovine serum albumin (BSA). Under the conditions used here, the mass spectrum of BSA in the absence of DDC and with no voltage difference applied between the nozzle and skimmer shows a very poorly resolved charge state distribution centered around the 13⁺ charge state (Figure 7a). Figure 7b shows the effect of the application of 34 V DDC, which shows the 14⁺ to 12⁺ charge states to be nearly baseline resolved. The application of a nozzle-skimmer potential of 200 V (Figure 7c) results in improved transmission through the interface, as reflected by the increase signal level, as well as significant declustering. However, the peaks are not as narrow as those in Figure 7b. The best result is obtained with a combination of both a 200 V nozzle-skimmer potential and 34 V_{DDC} (Figure 7d). The 15⁺ to 12⁺ peaks are the narrowest observed in all the experiments and show the highest signal-to-noise ratios.

CONCLUSIONS

The application of dipolar DC to opposing rods in a quadrupole array has been shown in previous work to be effective as a means for ion acceleration/collisional activation in linear ion traps operated in the presence of a bath gas. The broad-band nature of this ion acceleration approach and the relatively uniform levels of ion heating make it particularly appealing as a means for heating populations of ions across a relatively wide m/z range. Declustering charge state distributions of relatively high mass ions generated by ESI in a quadrupole array after transmission through the interface and prior to further transmission into a tandem mass spectrometer provides an independent means for processing cluster ions. Relatively precise control over ion heating is obtained via control over the V_{DDC}/V_{RF} ratio. The control of the time over which the DDC voltage is applied provides a measure of control over the extent of cluster dissociation for a given V_{DDC}/V_{RF} ratio. The upper m/z value, $(m/z)_{max}$, over which ion heating via dipolar DC can be effected is inversely related to the change in ion temperature, T_K , that can be achieved, as they have opposing dependences on the V_{DDC}/V_{RF} ratio. However, for a given V_{DDC}/V_{RF} ratio (i.e., constant T_K), the $(m/z)_{max}$ value can be increased via the use of higher V_{RF} values, at the expense of $(m/z)_{min}$. With the operating conditions used here, the DDC approach in a quadrupole array that samples ions that issue from the interface can be clearly useful for ions as high as m/z 10,000. The approach has been demonstrated to be a useful independent means for heating relatively high mass ions that can be used in addition to other commonly used approaches, such as ion desolvation techniques prior to sampling ions into the interface and ion heating techniques used within the interface.

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Scheme 1.

Typical scan function used for the application of ion trap DDC in the Q0 ion guide.

Figure 1. Q0 DDC CA applied to carbonic anhydrase cations trapped in Q0 with 300 V_{RF}. Sample contaminated with ubiquitin peaks which are labeled in red. (a) Q0 trapping time of 100 ms, no V_{DDC} applied. (b) Applying 34 V_{DDC} for 100 ms.

Figure 2. Effects of Q0 storage and Q0 DDC CA on declustering an RNA mixmer. (a) No Q0 storage. (b) 200 ms Q0 trapping. (c) 10 V_{DDC}, 263 V_{RF}, 100 ms in Q0, followed by 100 ms Q0 cooling.

Figure 3. DDC CA of pyruvate kinase tetramer. (a) 5 s trapping in Q0 with no DDC. (b) 200 V_{DDC} applied for 10 ms in Q0 during the 5 s trapping period.

Figure 4. 100 ms DDC CA of pyruvate kinase tetramer with fixed DDC amplitude (247 V) and variable RF amplitude. (a) 1607 V_{RF} (b) 1460 V_{RF} (c) 1315 V_{RF} .

Figure 5. 100 ms DDC CA of pyruvate kinase tetramer with fixed RF amplitude (1315 V_{RF}) and variable DDC amplitude. (a) 167 V_{DDC} (b) 205 V_{DDC} , (c) 239 V_{DDC} .

Figure 6. DDC CA of pyruvate kinase tetramers with variable heating time, 1607 V_{RF}, 212.5 V_{DDC}. (a) 10 ms (b) 50 ms (c) 200 ms (d) 1600 ms

Figure 7.

Comparison of BSA mass spectra under various nozzle-skimmer CA and DDC CA conditions Q0. (a) 0 V_{N-S} , 0 V_{DDC} . (b) 0 V_{N-S} , 34 V_{DDC} . (c) 200 V_{N-S} , 0 V_{DDC} . (d) 200 V_{N-S} , 34 V_{DDC} . In all cases, a 100 ms storage time in Q0 was employed.