Electron Capture Dissociation device in a branched RF ion trap on a QqToF platform with enhanced duty cycle

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ABSTRACT

A branched rf-ion trap ECD device [1] was coupled to a QqToF platform with novel ion trapping/releasing strategy termed “Zeno” [2]. The performance of the system has been evaluated on model peptides. Almost complete restoration of ToF duty cycle was achieved for ECD fragments yielding on average ~5.5x increase in sensitivity. No losses in mass accuracy have been detected.

INTRODUCTION

ECD and ETD are often implemented in high-throughput mass spectrometers with a particular advantage for PTM characterization and top-down analysis. In a typical ECD/ETD experiment only ~30-50% of precursor ions can be efficiently consumed without product neutralization. This poses even more strenuous requirements for the ion detection system than the conventional MS/MS strategy using CID. Typical QqToF mass spectrometer operates with duty cycle from 1% to 30% depending on its geometry and ion m/z. Recently, a novel ion trapping/releasing strategy termed “Zeno pulsing” has been proposed to address the issue, enabling almost complete recovery of these losses [2]. In this work a branched rf-ion trap ECD device [1] was coupled to an QqToF modified according to this strategy and its performance was evaluated.

PRINCIPLES OF ZENO ION TRAPPING/RELEASING STRATEGY

In conventional ToF mass spectrometer ions are continuously introduced to ToF accelerator region which leads to ion losses for ions having sufficient time to pass the accelerator while previous ion packet is flying towards the detector. This defines a duty cycle as: $Duty\ cycle(\%) = \frac{l}{b}\sqrt{\frac{m/_z}{(m/_z)_{max}}} * 100(1)$, where l is a size of accelerator, b is the distance between accelerator and detector, m/z is analyte mass to charge ratio and $(m/z)_{max}$ is the largest m/z acquired in spectra. To guide ions to the detector the axial and orthogonal energies need to be matched, where orthogonal energy is defined by ToF and independent of m/z. Pre-trapping and subsequent simultaneous release of ions with the same energy results in different arrival times to the center of ToF accelerator. Therefore any synchronization of ToF pulse with the ion release from the trap would lead only to amplification of signal in a narrow mass range. In order to achieve sensitivity enhancement across the whole mass range simultaneous ion arrival of ions with different m/z to the center of ToF accelerator with the same axial energy is required. Since the axial ion energy is constrained the only remaining parameter is the time when ions of certain m/z are released from the trap. A trap formed by AC pseudopotential barrier and attractive DC is sufficient to trap lighter ions and eject heavier ions in controlled manner. A strategy based on this property can be implemented for selective ion release from ‘Zeno’ trap (see Figure 1 bottom inset) and synchronization of their arrival to the center of the accelerator. For this an additional electrode named ‘Zeno’ gate (ZG) is installed at the end of collision cell to form a trap consisting of Q2 rods, IQ3 electrode and ZG. An additional AC potential is applied to all four rods of the Q2 to create a mass dependent pseudopotential barrier in the axial direction. With this arrangement ions can be trapped and released from the trap in a mass dependent way by applying appropriate potential. As shown in [2] a linear AC ramp is sufficient to focus ions in the center of the accelerator. A full ‘Zeno’ cycle consists of ion loading into the cell, ion cooling, mass dependent ion release from ‘Zeno’ trap and subsequent ToF extraction. Appropriate time diagram to accomplish such sequence is shown in Figure 2.

MATERIALS AND METHODS

All experiments were performed on a research grade X500B(SCIEX) mass spectrometer with shortened collision cell and a ‘Chimera’ ECD cell installed in front of it (see Figure 1). The ECD cell was controlled using custom-built electronics and software developed on LabView platform. The ECD cell was operated in quasi-flow through regime when ions were trapped and irradiated by electrons simultaneously. The detection system was upgraded to a 4-channel 10-bit 5GHz ADC (Cronologic). To restore TOF duty cycle a setup described above was used. An AC frequency was 2.5MHz and peak-to-peak voltage 1400V.
All chemicals were purchased from Sigma Aldrich.

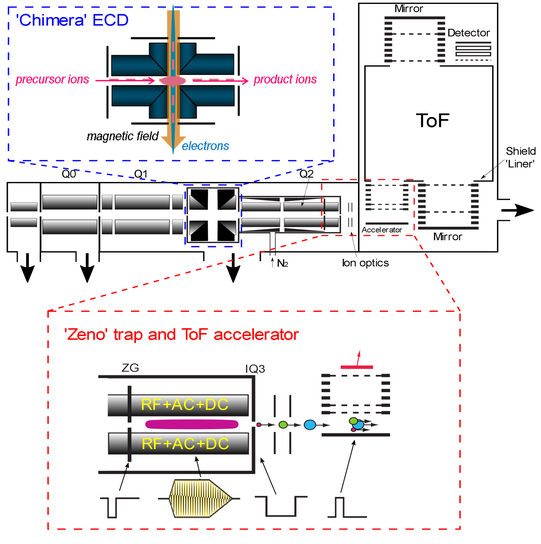

Figure 1. Schematic of modified ToF mass analyzer with ECD cell and ‘Zeno’ trap. Insets show principles of operation of ‘Chimera’ ECD cell and ‘Zeno’ trap respectively.

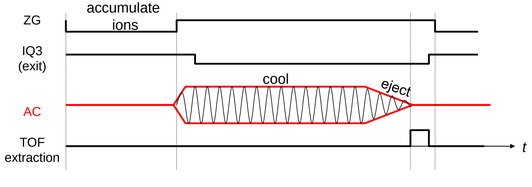

Figure 2. Timing diagram of ‘Zeno’ gating voltages, AC ramp

RESULTS

To evaluate the performance of the system an ECD spectra of Melittin $[M+4H]^{4+}$ were acquired both with ‘Zeno’ mode on and off (Figure 3). An ECD device was operated in simultaneous reaction and trapping mode at 100Hz frequency. Electron current and electron energy were optimized to achieve good fragmentation efficiency (>30%).

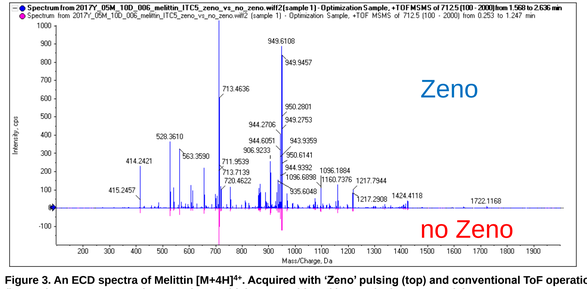

Figure 3. An ECD spectra of Melittin $[M+4H]^{4+}$. Acquired with ‘Zeno’ pulsing (top) and conventional ToF operation (bottom). A ~5.5x average increase in sensitivity was achieved by employing Zeno pulsing

Product ion intensities were further extracted from the same data file and experimental gain in sensitivity was compared to theoretical using relationship (1). The results are shown in Figure 4a. Overall >90% of theoretical gain was achieved for the sum of detected products yielding to ~5.5x increase in sensitivity on average. Slightly different gain was observed for c and z fragment series suggesting that ion processing during ‘Zeno’ cycle affects ECD reaction. Ion trapping during cooling phase and possible rf-excitation can potentially cause this effect.

In order to evaluate whether excessive excitation during ‘Zeno’ cycle can lead to unwanted CID fragmentation an experiment was performed where labile triply charged Substance P ion $[M+3H]^{3+}$ was analyzed using 3 different acquisition strategies and its fragmentation was monitored. In the first experiment standard experimental conditions were used (collision energy 10eV) and ECD cell was operated as a flow through device. In other two experiments the conditions were optimized to minimize fragmentation (collision energy 5eV) and ions were trapped in ECD cell mimicking ECD operation. Results are shown in Figure 5. Little excessive fragmentation has been found during ‘Zeno’ ion processing.

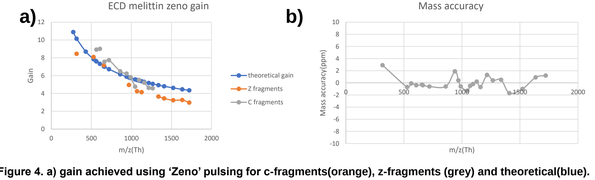

Figure 4. a) gain achieved using ‘Zeno’ pulsing for c-fragments(orange), z-fragments (grey) and theoretical(blue). b) Mass accuracy achieved in ECD experiment (Melittin [M+4H]4+) using ‘Zeno’ pulsing with total ion load 500000 cps and ECD device operating at 100Hz frequency.

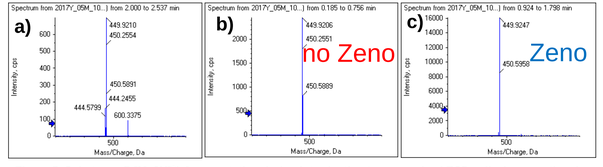

Figure 5. Substance P $[M+3H]^{3+}$ fragmentation during introduction to pressurized ECD cell. a) conventional operation with ECD device operating as flow-through device and collision energy 10eV, no ‘Zeno’ pulsing. b) gentle ion introduction into ECD cell (collision energy 5eV), ions are trapped in ECD cell, mimicking ECD operation, no ‘Zeno’ pulsing. c) same as b), but ‘Zeno’ pulsing on.

In ‘Zeno’ mode higher overall sensitivity and lower ToF pulser frequency (1.5kHz vs 10.5kHz) result in substantially higher number of ions per ToF extraction, which can lead to premature detector saturation. An ECD trapping device operating at even lower frequency 10-200Hz can further exacerbate the problem. Performance of the system was tested with total ion load of 500000 counts per second and ECD operating at 100Hz. A mass accuracy plot for selected fragments is shown in figure 4b with no adverse effects.

CONCLUSIONS

- **ECD device in a branched RF ion trap was coupled to ‘Zeno’ pulsing ToF instrument resulting in ~5.5x increase in sensitivity on average.**
- **For detected fragments no adverse effects on ToF mass accuracy were found with total ion count up to 500000 per second.**
- **Additional trapping and excitation during ‘Zeno’ cycle can affect ECD products promoting ion reactions.**
- **Additional ion excitation during ‘Zeno’ pulsing is not causing excessive fragmentation of labile ions.**

REFERENCES

[1] Baba T., Campbell L.J., Le Blanc Y.J.C., Hager J.W., Thomson B. A., Anal. Chem., 87, 785–792, 2015
[2] Loboda A.V. and Chernushevich I.V., J. Am. Soc. Mass Spectrom., v.20, 1342-48, 2009.

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