Increasing analytical separation and duty cycle with non-linear analytical mobility scan functions in TIMS-FT-ICR MS

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ABSTRACT: In this work, non-linear, stepping analytical mobility scan functions are implemented to increase the analytical separation and duty cycle during tandem Trapped Ion Mobility Spectrometry and FT-ICR MS operation. The differences between linear and stepping scan functions are described based on length of analysis, mobility scan rate, signal-to-noise, and mobility resolving power. Results showed that for the linear mobility scan function only a small fraction of the scan is sampled, resulting in the lowest duty cycle 0.5% and longest experiment times. Implementing non-linear targeted scan functions for analysis of known mobilities, resulted in increased duty cycle (0.85%) and resolving powers (R up to 300) with a six-fold reduction in time, from 30 to 5 minutes. For broad range characterization, a non-linear mobility stepping scan function provided the best sensitivity, resolving power, duty cycle (4%), and points per peak. The applicability of non-linear mobility scan functions for the analysis of complex mixtures is illustrated for the case of a direct infusion of a MCF-7 breast cancer cell digest, where isobaric peptides (e.g., DFTPAELR and TTILQSTGK) were separated in the mobility domain (R_{IMS}: 110) and identified based on their CCS, accurate mass (R_{MS}: 550k) and tandem MS using IRMPD in the ICR cell.

A common technical challenge faced when coupling traditional pre-separation technique (e.g., gas and liquid chromatography) with FT-ICR MS is that FT methods rely on measuring the free induction decay of ions over a period of seconds per scan in order to achieve ultrahigh mass resolution.[1,2] The ultra-high resolving power of FT-ICR MS instrumentation has unique advantages in the analysis of environmental,[3] biological,[4] and petroleum mixtures[5] compared to non FT methods. However, optimal operation of the FT-ICR MS (e.g., sensitivity and resolution) requires a well-defined ion population in order to minimize coalescence of the ion signal.[6-8] Several groups have shown the analytical advantages of coupling post-ionization separation techniques like ion mobility spectrometry (IMS) to FT-ICR MS. For example, the coupling of scanning IMS techniques (e.g., FAIMS)[9] to FT-ICR MS has been advantageous for the analysis of poly(ethyleneglycol),[10] proteins,[11-13] and glycans.[14] The coupling of time-dispersive IMS techniques (e.g., drift tube IMS) has also been shown for the separation of phosphopeptides[15] and to study ion-molecule reaction chemistry[16]. Alternatively, we have shown that time-independent IMS analyzers, such as Trapped Ion Mobility Spectrometry (TIMS)[17-19], can be effectively coupled to FT-ICR MS in selective accumulation[20], oversampling[21] and gated[22] modes of operation. These modes of operation have enabled mobility resolving powers up to 300 and mass resolution up to 1 million, giving the unique advantages when coupled to FT-ICR MS for the analysis of complex mixtures.[23-26]

In this work, for the first time, non-linear, stepping analytical mobility scan functions are implemented in gated TIMS to increase the analytical separation and duty cycle during tandem TIMS and FT-ICR MS operation. Following the concept of non-linear mobility scan steps used in TIMS-TOF MS,[27] the differences between linear and non-linear stepping scan functions are described based on length of analysis, mobility scan rate, signal-to-noise, and mobility resolving power. Three modes of operations (i.e., linear, nonlinear targeted and nonlinear stepping) are evaluated and their application illustrated for the mobility separation of a binary mixture of isobaric peptides in a complex mixture, as well as the potential for mobility selected tandem MS experiments.

EXPERIMENTAL SECTION

Materials and Reagents. A Tuning Mix calibration standard (G24221A) was obtained from Agilent Technologies (Santa Clara, CA) and used as received. Peptides DFTPAELR and TTILQSTGK were added at 10 μM to an MCF-7 digest[26] at 2.68 μg/μl and 10% of 0.1% methanol:formic acid.

TIMS and FT-ICR MS analysis. TIMS separation was performed using nitrogen as a bath gas at *ca.* 300 K, P_1 = 2.2-2.47 and P_2 = 0.9 mbar, and a constant V_{out} = 50 V and *rf* (840 kHz and 240-260 Vpp). The TIMS-FT-ICR MS experiments were acquired in chromatography mode, where each MS scan was a single 1-8 Megaword (0.5-4 second) transient, processed using a sine-squared apodization followed by fast-Fourier transform (FFT) in magnitude mode with an experimental resolving power of 50,000-550,000 at *m/z* 400. The individual scans are converted to mobility using the reported mobilities of hexakis fluorinated alkyl phosphazines found in the tuning mix mixture *m/z* 622 K_0=1.008, *m/z* 922 K_0=0.826, *m/z* 1222 K_0=0.711, *m/z* 1522 K_0=0.632 $cm^2V^{-1}s^{-1}$) more details can be found in the Supporting Information.

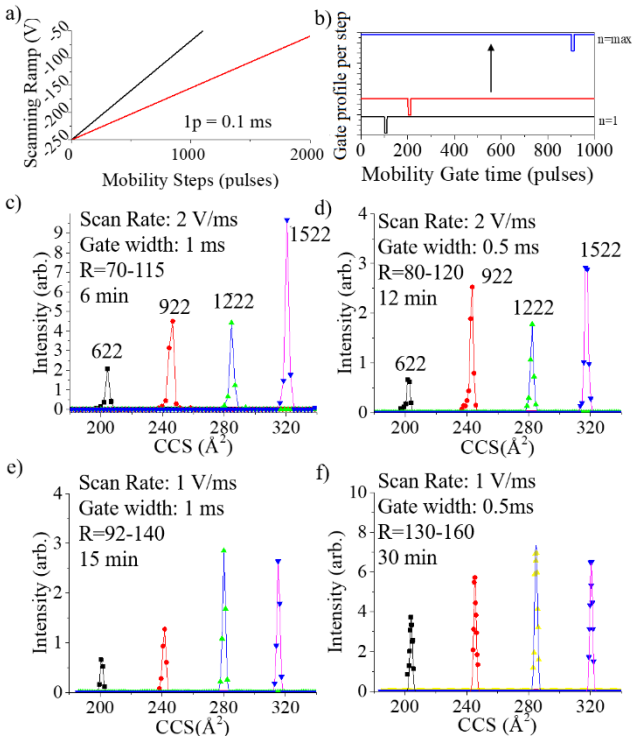

Figure 2. Typical linear scan TIMS profiles for Tuning Mix standard . (a) Scan over (ΔV_{ramp}=200V) and t_{ramp} of 100 and 200 ms. (b) Gate profile per step as a function of the gate time, letting subsequent portions of the ramp into the FT-ICR MS. (c) IMS profiles for t_{ramp}=100ms and 1 ms gate pulse, (d) IMS profiles t_{ramp}=100 ms and 0.5 ms gate pulse, (e) IMS profiles t_{ramp}=200 ms and 1 ms gate pulse, and (f) IMS profiles t_{ramp}=200 ms and 0.5 ms gate pulse. FT-ICR MS spectra were collected at 1 MW with 0.5s transient and R_{MS}~50,000 at m/z 400.

RESULTS AND DISCUSSION

In TIMS, ions are spatially resolved along the analyzer axis, in a time independent process where ions reach an equilibrium between the drag force caused by collisions with a moving gas and the increasing electric field as a function for their mobilities.[17-19] The electric field is established by a voltage gradient between the front (V_{ramp}) and the end (V_{out}) of the analyzer section, and stepwise reduction of V_{ramp} causes the ions to move towards the end of the analyzer in discrete mobility resolved packets. The TIMS experiment, which typically lasting between 100-500 ms, is traditionally coupled with a Time of Flight (ToF) mass analyzer, which has an experiment time of 100-150 µs, which is capable of quickly sampling the mobility separated ions as they elute from the TIMS analyzer. When combined with FT-ICR MS, which has experiment times on the order of seconds, the challenge becomes in achieving an analytically significant number of points across a mobility peak in a reasonable amount of time. When coupled to FT-ICR MS, the mobility range is sampled using discrete gating pulses, therefore, the number of points across the peak is dependent on the size of the elution steps. In a typical linear scan gated TIMS experiment, the mobility range, mobility resolution, analysis time and number of points across a mobility peak are defined by: scan rate, V_{ramp} range and gate width (see Figure 1). For example, for a V_{ramp}=200 V, t_{ramp}=100 ms and a 1 ms gate, the Tune Mix mobility peaks are characterized by just 3-4 points, from baseline, (i.e., 3-4 FT-ICR MS scans), which is too low for a true analytical characterization of an IMS peak profile (i.e., quantitative analytical characterization of a peak is typically defined as 15-20 points per peak). By reducing the gate width by half (1.0 to 0.5 ms), the number of points across the mobility peak increases from 3-4 to 6-8 peaks at the cost of doubling the analysis time (6 to 12 min). Another strategy to increase the number of points per peak, is to decrease the scan rate; for example, by doubling the ramp time from 100 to 200 ms over V_{ramp}=200 V with a gate width of 1 ms, the number of points across the mobility peak increases from 3-4 to 6-8 peaks in 15 minutes, with slightly higher mobility resolution (Fig 1e vs Fig 1d). If the gate width is then decreased from 1.0 to 0.5 ms, 10-12 points, from baseline, across the mobility are observed with higher resolving power (R=130-160). While the reducing the scan rate and gate width are effective methods for better peak profiles and higher mobility resolving power, they come at a cost of increasing the

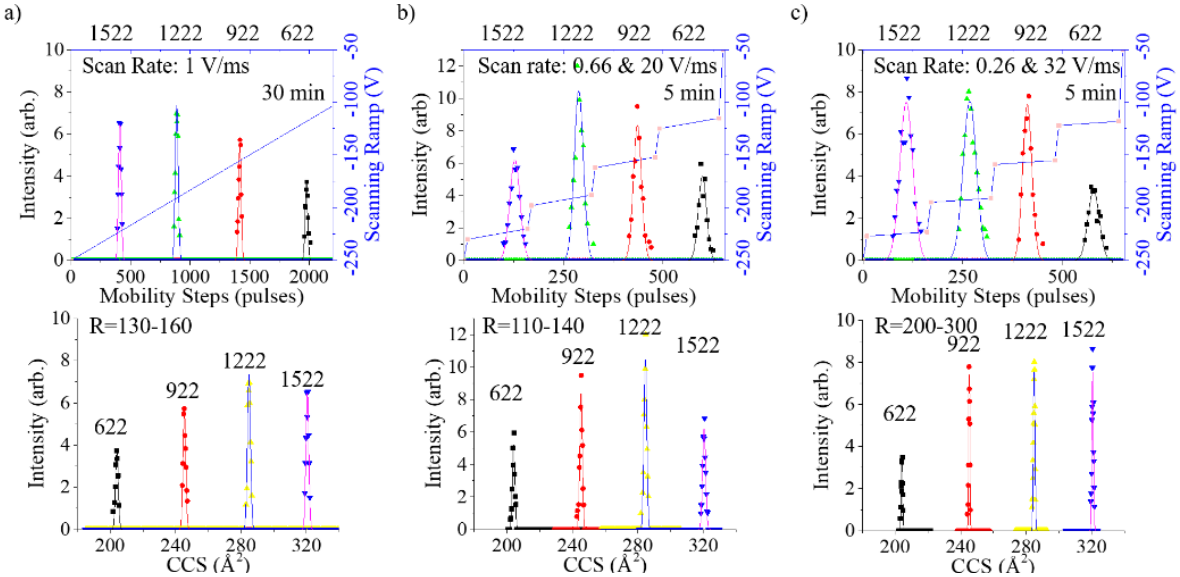

Figure 1. Comparison of the IMS profiles using a) linear scan TIMS (ΔV_{ramp} =200 V and t_{ramp}=200 ms) and non-linear scan TIMS with analytical ramps of b) ΔV_{ramp} =10 V and c) ΔV_{ramp} =4 V over 15 ms. For all experiments a 0.5 ms gate pulse was used. Top) Scanning ramp profiles are shown in blue and bottom) IMS profiles are shown in CCS. FT-ICR MS spectra were collected at 1 MW with 0.5s transient and R_{MS}~50,000 at m/z 400

experiment time and reducing the duty cycle (in this work duty cycle is calculated as the ratio of gate width to the total IMS time). For example, in the described TIMS-FT-ICR MS experiment a duty cycle of 0.5% is obtained for the t_{ramp}=200 ms, ΔV_{ramp}=200 V and 1.0 ms gate pulse width. That is, in this approach, the majority of the analysis time goes to scanning the V_{ramp} voltage, with only a small fraction of the scan steps allowed to be transmitted and accumulated in the FT-ICR MS

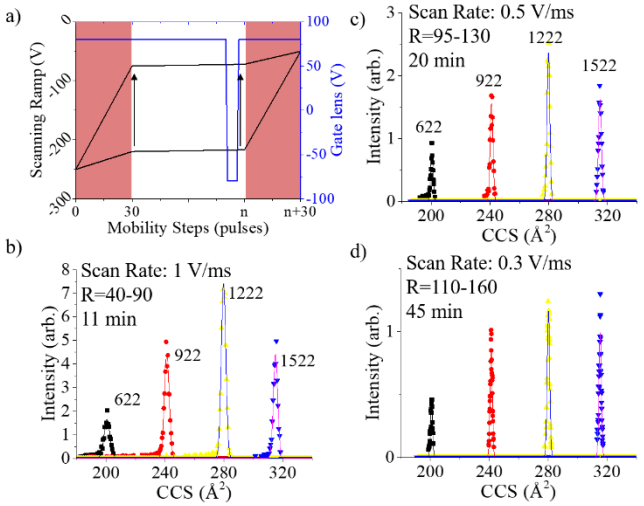

Figure 3. (a) Typical nonlinear stepping mobility scan TIMS experiment. The portions of the ramp highlighted in red are not sampled, and with each subsequent step the voltages of the analytical ramp are incremented, as shown by the black arrows, maintaining the same ΔV_{ramp}. Typical Tune Mix results are shown for an ΔV_{ramp}= 3 V scanned over (b) 3 ms, (c) 6 ms, and (d) 9 ms. FT-ICR MS spectra were collected at 1 MW with 0.5s transient and R_{MS}~50,000 at m/z 400

collision cell (defined by the gate width).

An alternative to this approach is to utilize *nonlinear targeted mobility scan functions* (nonlinear ΔV_{ramp}) where the scan rate is adjusted based on the mobility regions of interest using analytical (slow) and non-analytical (fast) scan rates (see Figure 2). This procedure is like a targeted quadrupole MS experiment where only specific masses are measured, and the others are skipped. For example, when performing a targeted analysis for ions at specific mobilities, the slower analytical portion of the ramp are centered on the targeted mobility, which is known beforehand. This approach not only reduces the analysis time but has the potential for even slower scan rates potentially leading to higher resolving power. For example, using a nonlinear scan rate function the Tune Mix analysis is reduced from 30 to 5 min using the same t_{ramp} and gate width and ΔV_{ramp} range with similar or higher resolving power (see Figure 2b and c). Note that the ratio between analytical and non-analytical can be varied as a function of the number of mobility peaks of interest without increasing the total experiment time. Under these conditions, the duty cycle is improved to 0.85%, but remains low since a narrow gate width is required to guarantee enough points across the mobility peak of interest.

In order to better correlate the timing of the analytical step in the TIMS profile with the gate width, a *nonlinear stepping scan function* can be designed such that only ions that are mobility separated are gated into the FT-ICR MS collision cell (see Figure 3). That is, a profile composed of a single analytical step can be used along with a gate at a fixed position. Subsequent mobilities are selected and separated by incrementing the voltages of the analytical step, changing the mobility region that undergoes high resolution separation (see Figure 3a). This approach does not require prior knowledge of an ions mobility, as in the previous approach, but allows the entire mobility range to be separated with the highest resolving power (i.e., using the slowest ramp speed across the analytical step), without exponential increases in time. For example, this nonlinear scan function with mobility stepping results in high resolving powers (up to 160), 26-30 points characterizing the peaks in the IMS profile, and better duty cycle (~4%) (Figure 3d). Another advantage of this approach is that *a priori* knowledge of the scan step of the peaks of interest is not necessary to define the analytical scan, since the full mobility range is acquired using slow scan rates (high mobility resolving power). A summary of the presented methodologies is provided in the supporting information

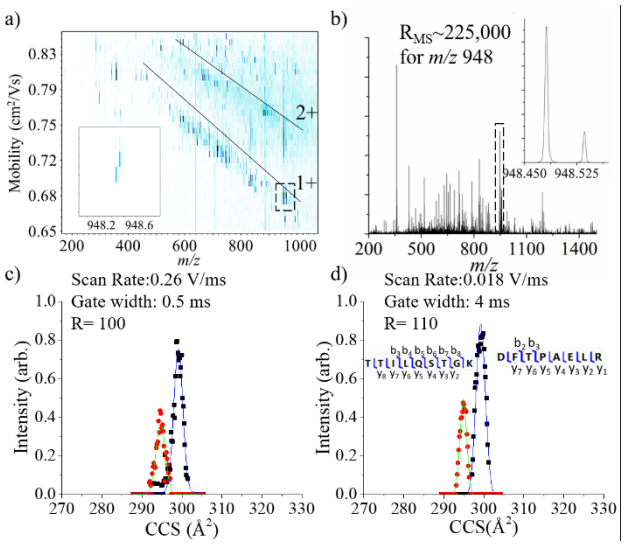

Figure 4. Analysis of isobaric peptides from a MCF-7 breast cancer digest sample. a) 2D-TIMS-FT-ICR MS contour plot acquired using a nonlinear stepping mobility scan function. b) typical MS spectrum for the MCF-7 digest with inset for the targeted peptide molecular ions. Typical mobility profiles for DFTPAELR (black squares and blue fit) and TTILQSTGK (red circles and yellow fit) using non-linear targeted (c) and stepping (d) mobility scan functions. FT-ICR MS spectra were collected at 8 MW with 4s transient and R_{MS}~550,000 at m/z 400.

The proposed methodology was applied for the targeted analysis of isobaric peptides from a MCF-7 breast cancer digest sample: DFTPAELR (*m/z* 948.47852) and TTILQSTGK (*m/z* 948.53603). Direct infusion of the MCF-7 breast cancer digest sample results in a highly complex MS spectrum. Taking advantage of the ultrahigh mass resolution of the FT-ICR MS, the signals corresponding to the DFTPAELR (*m/z* 948.47852) and TTILQSTGK (*m/z* 948.53603) can be easily separated with a resolving power of R_{MS}~225 000 at *m/z* 948. Moreover, the addition of the mobility dimension is particularly useful in analyzing these peptides because it enables complementary confirmations based on i) their collision cross section of the peptides and ii) tandem MS without the need of mass selection of the parent ion in the mass domain since they are separated in the mobility domain.[26]

The TIMS analysis using a linear scan function (t_{ramp} = 100 ms and gate width of 1 ms) lasted one hour and provided a detailed

characterization of the MCF-7 breast cancer digest sample and accurate CCS values of the two isobaric peptides (<2% error), but they were not resolved in the mobility domain (resolving power of 30). When non-linear scan functions are utilized the analysis time is reduced to 10 min (Figure 4c) and 35 min (4d), and the two peptides were resolved in the mobility domain (resolving power exceeding 100). Because the nonlinear scan function with mobility stepping has a greater duty cycle than the linear or multi-segments nonlinear functions, this mode can be utilized to increase the analysis sensitivity with multiple fills in the collision cells of mobility selected ion packets. Tandem MS of mobility selected ion packets using infrared multiphoton dissociation (IRMPD) in the ICR cell permitted further confirmation of the identities of DFTPAELR and TTILQSTGK with 100% sequence coverage, respectively (see tandem MS spectra in Figure S1). This example illustrates the potential of using nonlinear scan mobility functions for achieving high resolution mobility separation, better analytical profile (number of points across the mobility peak), accurate CCS measurements and increase duty cycle in tandem with ultrahigh MS and tandem MS separation. We anticipate that this powerful and flexible instrumental platform will find wide application in the analysis of complex mixtures in biomedical, environmental, and forensic applications.

CONCLUSIONS

Several modes of operation of a TIMS analyzer in tandem with FT-ICR MS were discussed. Differences in analysis time, resolving power, analytical profiles (number of points across the mobility peaks) and resolving power were shown between linear and two forms of nonlinear mobility scan functions: targeted and stepping. Results showed differences in duty cycles from up to 1% with the linear TIMS, to 5% using nonlinear functions with variable stepping TIMS. Compared to linear TIMS, the nonlinear functions are able to achieve greater resolving powers by decreasing the scan rate of the analytical step without increasing the total analysis time. The advantage of the proposed technology was successfully evaluated in a complex MCF-7 breast cancer digest sample for the separation and identification of isobaric peptides with mobility resolving powers exceeding 110, accurate CCS measurements (<1% error) and tandem MS using IRMPD in the ICR cell for sequence identification.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website. Additional experimental information, table s1 summarizes the experimental results, and figure S1 shows the IRMPD spectra of peptides DFTPAELR and TTILQSTGK.

The Supporting Information is available free of charge on the ACS publications website.

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REFERENCES

(1) Nikolaev, E. N.; Boldin, I. A.; Jertz, R.; Baykut, G. *J Am Soc Mass Spectrom* **2011**, *22*, 1125-1133.
(2) Benigni, P.; DeBord, J. D.; Thompson, C. J.; Gardinali, P.; Fernandez-Lima, F. *Energy & Fuels* **2016**, *30*, 196-203.
(3) Hertkorn, N.; Harir, M.; Cawley, K.; Schmitt-Kopplin, P.; Jaffé, R. *Biogeosciences Discussions* **2015**, *13*, 2257-2277.
(4) Xie, Y.; Zhang, J.; Yin, S.; Loo, J. A. *J Am Chem Soc* **2006**, *128*, 14432-14433.
(5) Klein, G. C.; Kim, S.; Rodgers, R. P.; Marshall, A. G.; Yen, A. *Energy & Fuels* **2006**, *20*, 1973-1979.
(6) Comisarow, M. B.; Marshall, A. G. *Chem Phys Lett* **1974**, *25*, 282-283.
(7) Marshall, A. G.; Hendrickson, C. L. *Int J Mass Spectrom* **2002**, *215*, 59-75.
(8) Qi, Y.; O'Connor, P. B. *Mass Spectrom Rev* **2014**, *33*, 333-352.
(9) May, J. C.; McLean, J. A. *Anal Chem* **2015**, *87*, 1422-1436.
(10) Robinson, E. W.; Garcia, D. E.; Leib, R. D.; Williams, E. R. *Anal Chem* **2006**, *78*, 2190-2198.
(11) Robinson, E. W.; Leib, R. D.; Williams, E. R. *J Am Soc Mass Spectrom* **2006**, *17*, 1469-1479.
(12) Robinson, E. W.; Sellon, R. E.; Williams, E. R. *Int J Mass Spectrom* **2007**, *259*, 87-95.
(13) Robinson, E. W.; Williams, E. R. *J Am Soc Mass Spectrom* **2005**, *16*, 1427-1437.
(14) Kailemia, M. J.; Park, M.; Kaplan, D. A.; Venot, A.; Boons, G. J.; Li, L.; Linhardt, R. J.; Amster, I. J. *J Am Soc Mass Spectrom* **2014**, *25*, 258-268.
(15) Tang, X.; Bruce, J. E.; Hill, H. H., Jr. *Rapid communications in mass spectrometry : RCM* **2007**, *21*, 1115-1122.
(16) Bluhm, B. K.; Gillig, K. J.; Russell, D. H. *Rev Sci Instrum* **2000**, *71*, 4078.
(17) Fernandez-Lima, F. A.; Kaplan, D. A.; Park, M. A. *Rev Sci Instrum* **2011**, *82*, 126106.
(18) Fernandez-Lima, F.; Kaplan, D. A.; Suetering, J.; Park, M. A. *Int J Ion Mobil Spectrom* **2011**, *14*, 93-98.
(19) Hernandez, D. R.; Debord, J. D.; Ridgeway, M. E.; Kaplan, D. A.; Park, M. A.; Fernandez-Lima, F. *The Analyst* **2014**, *139*, 1913-1921.
(20) Benigni, P.; Thompson, C. J.; Ridgeway, M. E.; Park, M. A.; Fernandez-Lima, F. *Anal Chem* **2015**, *87*, 4321-4325.
(21) Benigni, P.; Fernandez-Lima, F. *Anal Chem* **2016**, *88*, 7404-7412.
(22) Ridgeway, M. E.; Wolff, J. J.; Silveira, J. A.; Lin, C.; Costello, C. E.; Park, M. A. *Int J Ion Mobil Spectrom* **2016**, *19*, 77-85.
(23) Benigni, P.; Sandoval, K.; Thompson, C. J.; Ridgeway, M. E.; Park, M. A.; Gardinali, P.; Fernandez-Lima, F. *Environ Sci Technol* **2017**, *51*, 5978-5988.
(24) Benigni, P.; Marin, R.; Sandoval, K.; Gardinali, P.; Fernandez-Lima, F. *J. Vis. Exp.* **2017**, *121*, e55352.
(25) Benigni, P.; Bravo, C.; Quirke, J. M. E.; DeBord, J. D.; Mebel, A. M.; Fernandez-Lima, F. *Energy & Fuels* **2016**, *30*, 10341-10347.
(26) Garabedian, A.; Benigni, P.; Ramirez, C. E.; Baker, E. S.; Liu, T.; Smith, R. D.; Fernandez-Lima, F. *J Am Soc Mass Spectrom*, (2017). https://doi.org/10.1007/s13361-017-1787-8.
(27) Silveira, J. A.; Danielson, W.; Ridgeway, M. E.; Park, M. A. *Int J Ion Mobil Spectrom* **2016**, *19*, 87-94.