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Perturbation-induced high-frequency pulsing of nano-ESI with facile ion selection at atmospheric pressure

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Abstract

Nano-ESI is a commonly used ionization technique with continually expanding analytical advantages. Here, we report a facile way for high-frequency (500–3800 Hz) pulsing of nano-ESI, providing a high flux of mobility-selected ions. The pulsing is accomplished using a relatively low-voltage modulation (80 V peak-to-peak) of an electrode placed < 1 cm downstream of a nano-ESI emitter biased to a constant potential. Configuring the electrode as an ion gate enables mobility-based ion selection by scanning the modulation frequency. Our investigations indicate that the electrode modulation perturbs continuous nano-ESI, resulting in solution accumulation at the emitter tip between spray pulses. Selective transmission of ions occurs at frequencies corresponding to harmonics of a fundamental frequency determined by the travel time of each ion from the emitter to the ion gate (pulsing electrode). Remarkably, the intensities of ions selected in this fashion are similar across the harmonics, suggesting that the ionization efficiencies of analytes have minimal dependence on the accumulated volume at the emitter tip. Moreover, intensities of ion-mobility-selected analytes using this technique reach > 50% of those in continuous nano-ESI without ion selection, underscoring efficient ion generation via high-frequency pulsing. These findings indicate the potential of the pulsed nano-ESI for enhanced analytical utility, such as a high-flux selected-reagent-ion supplier at atmospheric pressure, and chart new avenues to further enhance the analytical performance of nano-ESI.

Graphical Abstract

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Conflicts of Interest

Authors do not have any conflicts of interest to declare.

A new nano-ESI pulsing regime allows selection of ions with minimal loss of ion flux compared to continuous spray.

Introduction

Nano-electrospray ionization (nano-ESI) offers improved sensitivities compared to electrospray operated at higher flow rates, partly because of smaller initial charged droplets.^{1–3} This improved performance has sparked continuous developments in nano-ESI. For example, recent studies using submicrometer emitters have shown reduced matrix effects, such as metal adduction, in nano-ESI.^{4,5} Pulsed operation via externally triggering ion production is another approach investigated to enhance the performance of nano-ESI. Various pulsing schemes have been utilized such as a piezoelectric-assisted pulsing,⁶ induced pulsing through a dielectric,^{7–9} pulsing the potential supplied to the solution,^{10–13} and pulsing the electric field in the proximity of the emitter.^{14,15}

Practical and fundamental advantages have been demonstrated for pulsed nano-ESI relative to continuous operation. For example, several studies have shown reduced sample consumption in pulsed mode relative to continuous operation.^{6,7,10,16} Moreover, heating of the emitter tip, deformation by electrical discharge, and clogging of the emitter are minimized in pulsed mode, facilitating the long-term use of nano-ESI tips.^{7,17} Importantly, pulsed nano-ESI has also shown promise in enhancing ionization. In one study comparing continuous nano-ESI operated at 150 nL/min to 20 Hz pulsed operation of the same source, 3.6 fold higher protonated reserpine intensities were detected in the pulsed mode.¹¹ Furthermore, 2–30 times higher signal-to-noise ratios have been reported for various analytes using a pulsed nano-ESI compared to continuous nano-ESI.^{11,16} Lastly, a temporal evolution of ions from a pulsed nano-spray has been demonstrated which could offer preferential tuning of nano-ESI for the detection of desired ions.¹²

Controlled pulsing of nano-ESI and short ion bursts also enable the direct use of this ionization mode for introducing discrete packets of ions into drift tubes utilized in atmospheric-pressure (AP) ion mobility separations.^{14,15} In a recent study using this approach, we investigated the effect of nano-ESI pulse duration on ionization, demonstrating that long pulse duration preferentially enhanced the intensity of low-ionization-efficiency

analytes, while the collision cross sections did not change appreciably. These studies indicated a temporal evolution of droplet sizes during a pulsed spray and suggested that final ion structures emerge rapidly with minimal impact of pulse duration on the measured collision cross section.¹⁵

The enhanced ionization using pulsed nano-ESI along with AP mobility-based ion selection opens new avenues for improving analytical techniques. For example, the approach may be used to supply reagent ions with rapid switching for selective and enhanced ionization.¹⁸ Such a capability, however, requires a high ion flux from the ion source. Our previous investigations using pulsed nano-ESI with AP mobility separations utilized relatively low frequencies (20–25 Hz), limiting the ion flux.^{14,15}

Herein we present a new approach for pulsing nano-ESI coupled with ion selection operated at high repetition rates (500–3800 Hz) to increase the ion flux. The approach is based on a simplified instrumental setup consisting of a nano-ESI and a single pulsing ion gate that perturbs nano-ESI operation at high frequencies, resulting in pulsation of the spray. We investigate this spray pulsing mechanism and compare the resulting ion flux to that in continuous nano-ESI to decipher the important factors for efficient ion generation in the pulsed mode. Further, we examine mobility-based ion selection capacities using this approach. These experiments highlight the potential of pulsed nano-ESI to enhance the analytical performance of nano-ESI-MS and to enable new analytical applications such as a selected-ion supplier at atmospheric pressure.

Experimental

Nano-ESI coupled to pulsing ion gate and MS

The experimental setup is depicted in Figure 1. A 5-cm long borosilicate glass nano-ESI emitter (i.d.: 5 ± 1 μm , WPI, Sarasota, FL, USA) was filled with analyte solution and connected to an analyte reservoir positioned 8–10 cm above the emitter using a 55-cm long fused silica capillary (i.d.: 100 μm), inducing gravity-assisted solution delivery to the emitter. A platinum electrode (0.25 mm dia., 99.997%, Alfa Aesar, Ward Hill, MA, USA) was inserted into the sample reservoir to supply a constant voltage (0.9–2.4 kV) to the solution.

The emitter was centered within a printed circuit board (PCB, DirtyPCBs, Shenzhen, China) ion guide housing based on a recently reported design for ion mobility cells.¹⁹ Briefly, the 2-cm-long ion guide consisted of two parallel PCBs (W: 4.86 cm, thickness: 0.16 cm) sandwiching 6 resistively coupled PCB ring electrodes (o.d.: 3.18 cm, i.d.: 2.50 cm, thickness: 0.16 cm) that were evenly separated by two 1-mm-thick non-conductive PCB spacers. A potential of 500–700 V was supplied to the first electrode (E1, see Figure 1) to guide the ions toward a three-mesh-grid ion gate.

The ion gate was constructed using stainless steel mesh (#28, Type B, E-Fab, Santa Clara, CA, USA) in a 3-grid shutter configuration similar to the ion gate described in other reports.^{19–21} The mesh was cut into three separate grids with the first grid (G1) placed in electrical contact with the downstream end of the last PCB electrode. A PTFE spacer (thickness: 300

μm) was inserted at the downstream end of G1, followed by grid 2 (G2), a PTFE spacer, and grid 3 (G3). The three stacked grids were aligned by inserting stainless steel rods through the mesh-grid openings during the assembly to maximize the overlap of mesh-open spaces. G1 potential was equal to the potential of the last PCB electrode, while G3 was grounded. G2 was electrically isolated and was pulsed using a voltage amplifier (piezo driver, Model: PZD350A, Trek, Lockport, NY, USA).

The PCB housing was then interfaced to a TOF-MS equipped with a glass ion transfer capillary (AxION 2 TOF, PerkinElmer, Waltham, MA, USA) using a home-built Delrin adapter (see Figure 1), positioning G3 approximately 4.7 mm upstream of the MS inlet. In positive mode, the MS ion transfer capillary inlet and shield were biased to -100 V , as shown in Figure 1, to attract the ions into the MS, while the capillary exit and skimmer were operated at $+40\text{ V}$ to minimize ion activation and to guide ions into the RF hexapole for ion transmission. Negative mode analysis used the same potential values biased to the opposite polarity.

The nitrogen counter flow introduced into the MS inlet was controlled externally with a flow meter (Matheson 603, Montgomeryville, PA, USA) and was optimized for stability of the ion flux with approximately 7 LPM entering the MS instrument unless otherwise specified. The gas flow rate entering the ion guide area from the MS was lower than that introduced into the MS interface because of the existence of other flow paths to the atmosphere within the interface. The design of these paths prevented a measurement of the actual flow rate in the ion guide region. Therefore, ion intensities and stabilities were used as metrics to optimize the gas flow rates for experiments.

To pulse G2, a microprocessor (Arduino Due, Torino, Italy) provided the pulse timings to an arbitrary waveform generator (Dataman-530, Orange City, FL, USA) which supplied the pulse shapes to the voltage amplifier. Ion arrival curves were generated by scanning pulse periods at increments of $5\text{--}20\text{ }\mu\text{s}$, while MS spectra were acquired at an acquisition rate of 1 Hz. During the scan, three to four spectra were collected per pulse period to improve signal-to-noise and to enhance quantitative comparisons. We note that the experimental time for each T sweep depends on scan parameters and is $4.75\text{--}16.07$ minutes for the experiments in this report. For example, a scan of $200\text{ }\mu\text{s}$ – $2000\text{ }\mu\text{s}$ with a step size of $10\text{ }\mu\text{s}$, MS acquisition rate of 1 Hz, and three spectra per T requires 9.05 minutes.

Chemicals and reagents

Unless otherwise specified, solutions were made using $18\text{ M}\Omega$ water as solvent and contained three tetraalkylammonium (TAA) ions: $3\text{ }\mu\text{M}$ tetraethylammonium ($[\text{TEtA}]^+$), $2\text{ }\mu\text{M}$ tetrabutylammonium ($[\text{TButA}]^+$), and $3\text{ }\mu\text{M}$ tetraheptylammonium ($[\text{THepA}]^+$) ions. Details for other analytes, sample preparation, and sources for reagents are provided in supporting information.

Results and discussion

Nano-ESI pulsing by ion gate modulation

A continuous nano-ESI was first used in our experiments to verify the operation of G2 as an ion gate (Figure 1). We note here that continuous operation in these experiments refers to any nano-spray mode that is not synchronously modulated by an external trigger. A 5- μm nano-ESI tip positioned 8.7 mm upstream of G1 (see Figure 1) and biased to +1339 V produced a stable and continuous stream of ions detected by the MS when the ion gate was set to an open state (G1= +100 V, G2= +50 V, G3= 0 V). No ions were detected by setting the gate to a closed state (G2= +132 V), indicating complete blockage of ions. Interestingly, when the G2 gate was pulsed between the two open/closed states (inset of Figure 2) at high frequencies, selective transmission of ions through the ion gate was observed by scanning the pulse period ($T=260\text{--}2000\text{ }\mu\text{s}$, frequency=500–3846 Hz) as shown in Figure 2. Moreover, the optimum period for ion detection, and the number of peaks observed for each analyte in this scan window were ion-dependent.

The relationship between the timings of the observed peaks in Figure 2 for each analyte provides insight into the origins of this observation. For each analyte in Figure 2, the times corresponding to the apex of peaks are labeled with pulse period values (T) using T_n notation where $n=0$ denotes the peak observed at the longest period while subsequent n values refer to the peaks observed at smaller T values. For example, no peaks were observed for $[\text{TButA}]^+$ beyond $1100\text{ }\mu\text{s}$. Thus, the peak at $1080\text{ }\mu\text{s}$ is labeled as T_0 for this ion, while the earlier peaks have successively larger n values. Notably, the T_n values for each ion appear to be related by equation 1:

$$T_n = \frac{T_0}{n + 1} \quad (\text{eq. 1})$$

Figure 3 demonstrates this relationship by plotting T_0/T_n as a function of n , allowing representation of all analytes from Figure 2 on a single line. The slope and intercept in Figure 3 are close to unity as expected from equation 1. This relation suggests that the earlier arriving peaks are harmonics of the last arriving peak for each ion. Thus, the last arriving peak defines a fundamental frequency ($f_0 = 1/T_0$).

These observations resemble the effects in frequency-coded ion transmission in ion mobility spectrometry with synchronously operated entrance and exit gates.^{22–25} Accordingly, we infer that pulsing G2 induces a synchronous pulse in the nano-ESI, and only ions that fully traverse the tip-to-gate distance at the fundamental period are allowed to pass through the gate.

Analyte accumulation at the emitter tip in between spray pulses

To further clarify the selective ion transmission in Figure 2 and to highlight the unique aspects of the gate-induced pulsed nano-ESI, we conducted a series of simulations using R language.²⁶ These simulations considered both continuous and pulsed operation of nano-ESI for comparison with the experimental results in Figure 2.

Figure 4a shows the scenario for a continuous nano-ESI unaffected by the gate voltage modulation at 2 kHz ($T = 500 \mu\text{s}$). The ion flux from the nano-ESI is simulated by a constant ion density at the gate (horizontal dashed blue line at unity) at all times. A gate pulse width (open duration) of $100 \mu\text{s}$ is considered (red trace) similar to the experiments in Figure 2. For simplicity, the ion transmission efficiency through the gate is modeled with a square pulse between 0 (no ion transmission when G2 is closed) and 1 (maximum transmission when G2 is open). The temporal ion density downstream of the gate is then calculated via multiplying the ion density trace at the gate (blue line) by the gate transmission efficiency (red line), resulting in the ion detection trace (green line) in Figure 4a. Note that ions are always present at the gate in this scenario, but they would only pass through the gate at times the gate is set to 1 (open state). Thus, the ion detection trace matches the gate transmission trace. To model the ion intensities observed by the MS at each T , an ion detection trace spanning one second was generated with a resolution of $1 \mu\text{s}$. The y-axis values (0s and 1s) were summed across the 1-second trace, reflecting the total ion intensity resulting from a 1-second MS acquisition time.

Figure 4b shows the simulated MS ion intensities as a function of the ion gate pulse period (T) for a continuous nano-ESI. A steadily decreasing ion intensity with $1/T$ dependence is evident in Figure 4b in contrast to the distinct peaks observed experimentally in Figure 2. The decreasing trend in Figure 4b stems from using a constant gate open duration (pulse width = $100 \mu\text{s}$) regardless of pulse period (T). Thus, longer T s lead to proportionally lower duty cycles for ion transmission through the gate, leading to a $1/T$ ion intensity dependence.

We now consider a scenario where the G2 pulse induces a pulsation in the nano-ESI operation. Pulsation of the nano-ESI could result from penetration of the electric field from G2 into the area upstream of G1 (see Figure 1). In the experiments for Figure 2, the gate opens when G2 is lowered from +132 to +50 V. Lowering of the gate voltage would enhance the electric field in the nano-ESI compartment. Thus, in our simulations we assume that a pulse of ions from nano-ESI is generated synchronously with the falling edge of the gate voltage pulse. Figure 4c illustrates the pulsed operation of both the gate and nano-ESI. The ion transmission through the gate (red trace) is simulated similar to that in Figure 4a. The pulsed nano-ESI is modeled using a square pulse with 0 representing no ion production and 1 representing the maximum ion density for each analyte generated synchronously with the rising edge of the gate transmission pulse (falling edge of the gate voltage pulse). For simplicity, an ion pulse width equivalent to the gate pulse width ($100 \mu\text{s}$) is also assumed in the model. We note that this assumption does not have any bearing on the results described below.

Notably, the packet of ions generated by the pulsed nano-ESI has to traverse the tip-to-gate distance prior to transmission through the gate. To simulate arrival of ions at the gate, we shifted the ion production trace (synchronized with gate transmission) by the tip-to-gate travel time of the ions. An example of simulated ion density trace at the gate is shown in Figure 4c (dashed blue line) using a $1080\text{-}\mu\text{s}$ shift, analogous to the travel time of $[\text{TButA}]^+$ in Figure 2. Notably, Figure 4c illustrates a pulse period of $T = 500 \mu\text{s}$ ($f = 2 \text{ kHz}$). Thus, ion packets spaced by $500 \mu\text{s}$ arrive at the gate, while each packet is generated synchronously with a gate pulse occurring $1080 \mu\text{s}$ earlier than its arrival. As noted above, multiplying the

gate transmission trace (red line) by the ion density trace (blue line) produces an ion detection trace (green line) at each T. The MS ion intensities are then simulated by summation of all values in a 1-second ion detection trace for each T.

Figure 4d shows the simulated MS ion intensities as a function of T using a synchronously pulsed nano-ESI for an ion that traverses the tip-to-gate distance in 1080 μs . The simulations resemble the experimental results in Figure 2 in regards to peak timings and peak widths. At the pulsing period of 1080 μs , the ion packet arrival is synchronized with the gate opening, resulting in maximal transmission of ions and the appearance of a peak centered at 1080 μs . Slightly lower or higher periods lead to the arrival of ions at the gate when the gate is closed, thus ion transmission is reduced (partial overlap of ion packet and transmission) or blocked. The model also produces harmonic peaks at periods corresponding to $T_n = 1080/(n+1)$, with $n=0-2$ shown in Figure 4d, and depicts narrowing peak widths with increasing n similar to experimental observations in Figure 2. The peak width narrowing with n results from scanning the gate transmission window across the ion packet at different frequencies. For example, the simulated ion packet in Figure 4d has a travel time of 1080 μs and a constant ion packet width of 100 μs . Using a 100- μs gate transmission window centered at 1080 μs (T_0), the entirety of the ion packet is transmitted, giving rise to a maximum intensity. When T is increased to 1085 μs , 95% of the ion packet is sampled. The transmission efficiency decreases linearly with T as the window is shifted farther, eventually reaching 0% when the center of the window is at 1180 μs . In other words, the ion packet and ion gate pulse have a total possible overlap width of 200 μs (100 μs in each direction) around T_0 for ion transmission. For the T_1 peak in Figure 4d, the 100 μs transmission window is centered at 540 μs , sampling the entirety of the ion packet generated two pulses prior to gate opening. However, when T is set to 545 μs (a 5 μs shift analogous to the T_0 case) the time difference between every other gate opening becomes $2 \times 545 = 1090$ μs , while the ion packet travel time to the gate remains a constant 1080 μs . In other words, the effective shift of the transmission window is 2 times larger than the 5- μs change, leading to a faster reduction in intensity as a function of T and a total possible overlap width of 100 μs (50 μs in each direction) for ion transmission around T_1 . In general, the effective time shift around T_n is $n+1$ times larger than the T, yielding a $n+1$ fold narrower peak at T_n compared to that at T_0 .

The simulation in Figure 4d, however, shows markedly different relative peak heights between harmonics compared to that observed experimentally in Figure 2. In simulations, intensities of the peaks increase linearly with increasing n. In other words, the peak at T_1 (appearing at $T_0/2$) has a factor of 2 higher intensity than the peak at T_0 in Figure 4d. This is a result of more pulses generated per unit of time at higher n values (corresponding to higher frequencies). In contrast, the experimental intensities of peaks for each ion in Figure 2 do not show a significant dependence on n. This observation suggests that more ions are detected per ESI pulse at smaller n values (larger T_n values, lower frequencies), compensating for the fewer pulses per unit of time. Such a behavior can be produced by analyte accumulation at the emitter tip between spray pulses and subsequent ionization of the accumulated analyte with a constant efficiency regardless of the accumulation time. In this mechanism, a constant analyte accumulation rate would increase the total ionized analyte in each pulse proportionally with pulse period (T), cancelling out the $1/T$ effect from

duty cycle considerations. Solution accumulation at the emitter tip and solvent evaporation are contributing factors to this mechanism and will be discussed in detail, later in the report.

Spray induction mechanism and ion flux examined by gate pulse shape

To experimentally verify spray induction by the falling edge of the gate voltage pulse and to improve the flux of mobility-selected ions, we investigated the effect of gate pulse shape on ion arrival curves (ion intensity as a function of T , similar to Figure 2). The three-grid gate offers several modes of operation.^{19–21,27,28} We started with two distinct modes shown by the insets of Figure 5, labeled as dual-state and reverse dual-state. The dual-state pulse is identical to the pulse shape shown in Figure 2, while the reverse dual-state pulse operates by blocking ions at -32 V (confirmed using continuous operation of nano-ESI) and transmitting at $+50$ V. Note that G3 is always held at ground. Therefore, setting a G2 = -32 V creates a potential barrier for positive ions between G2 and G3, preventing them from passing through the gate. The resulting ion arrival curves using the two pulse modes at a constant $100\text{ }\mu\text{s}$ gate pulse width (gate open duration) are shown in Figure 5a for [TButA]⁺.

As illustrated in Figure 5a, the pulse shape has a major impact on the ion arrival curves. Three differences are immediately apparent between the two pulse modes: (1) peak heights (flux of mobility-selected ions) are significantly higher using the reverse dual-state pulse, (2) peak shapes are more symmetric with the reverse dual-state operation, and (3) peaks are observed at a later time for reverse dual-state operation compared to those in dual-state operation. The improvements in peak shape and peak heights have contributions from more efficient ion gate operation in the reverse dual-state mode. This is further clarified in Figures 5b, 5c, and 5d where zoomed-in areas for T_0 peaks of three TAA ions are illustrated. The peak height ratios between the two operating modes follow a trend of 2.1, 2.6, and 6.1 for [TEtA]⁺, [TButA]⁺, and [THepA]⁺, respectively, favoring the reverse dual-state operation. The greater improvement for the low-mobility TAA ions indicates that mobility-based ion discrimination effects at the gate are reduced in the reverse dual-state mode, consistent with effects observed in other reports.²⁷

The difference in ion arrival times between the two modes in Figure 5a relates to the spray induction timing. Notably, the difference is amplified by increasing the gate pulse width from $100\text{ }\mu\text{s}$ to $150\text{ }\mu\text{s}$ as shown in Figures 5 b–d (see the dashed lines in insets of Figure 5a for pulsing diagrams). A longer gate pulse width in regular dual-state operation shifts the peaks to earlier times. In contrast, a longer pulse width increases the arrival time of ions using the reverse dual-state pulse scheme. These opposite trends have different origins and shed light on the pulsed spray induction mechanism as discussed below.

In both modes, the pulse period reflects the time between two instances of gate opening (see Figure 5a inset). If the spray is induced by the gate closing, increasing the gate pulse width delays the timing of the spray generation relative to the beginning of the period and shortens the time difference between the spray formation and gate opening in the next pulse (see reverse dual state in inset of Figure 5a). Accordingly, the pulse period must be elongated to observe the ion arrival peak apex corresponding to gate opening at the ion packet arrival time. Further, the same amount of time shift in pulse period is expected for all ions upon increasing the pulse width. Notably, similar positive shifts for all ions are observed in

reverse dual state mode in Figures 5b–d upon increasing the pulse width, implying that nano-ESI pulse is induced with gate closing in this mode.

In contrast, if the spray is induced by the gate opening, increasing the gate pulse width would not change the spray formation time relative to the gate opening time in the next pulse. Therefore, no significant change in the pulse period for maximum ion intensity (arrival peak apex) is expected. We posit that the regular dual state mode operates in this regime, but the negative time shifts with increasing pulse width have a different cause evident from the trends in Figure 5b–d. In regular dual-state mode, the peak shifts to earlier times and improvements in peak heights upon increasing the gate pulse width (Figures 5b–d) become more pronounced for ions with lower gas-phase mobilities. This trend suggests the involvement of mobility discrimination effects at the gate in the time shifts to earlier values. Notably, mobility discrimination effects are minimized using reverse dual-state pulse (further evident from the lower impact of pulse width on peak heights in Figure 5d), suppressing analyte-dependent time shifts in this mode upon changes in pulse width.

In summary, the results indicate that the nano-ESI pulse is induced by gate opening in regular dual-state mode and by gate closing in reverse dual-state mode. Importantly, these spray induction timings correspond with the falling edge of the gate voltage pulse in both modes, confirming that the nano-ESI is modulated by a momentary field enhancement induced by the gate operation.

A third gate pulsing mode, tri-state operation, has been shown to further reduce ion discrimination effects using three-shutter ion gates.^{27,28} To investigate the potential advantages of this mode with pulsed nano-ESI, we compared the tri-state mode and reverse-dual state operation. The pulsing schemes for the two modes are shown in Figure S1a with a constant total gate pulse width (100 μ s) applied in both modes. The tri-state pulse includes a 20 μ s push duration to enhance the transmission of ions admitted into the gate area during the gate open time (80 μ s).

The resulting ion arrival curves are shown in Figures S1b–d, comparing reverse dual-state and tri-state gate operations. Notably, the arrival times for the ions are similar between the two modes, indicating that pulsing of the nano-ESI is also triggered at the falling edge of the tri-state pulse. Furthermore, peak heights are similar between the two modes for all three TAA ions, suggesting that ion discrimination effects are not reduced beyond that observed for the reverse dual-state operation. This could be a consequence of the relatively long gate pulse widths used in our experiments and would be explored in future studies.

Overall, the various pulsing schemes confirm the generation of a spray induced by the falling edge of the gate voltage pulse and highlight the importance of the gate operation on the flux of mobility-selected ions and peak shapes, which impact ion selection resolution.

Perturbation-induced pulsing of nano-ESI: an effect observed in high-frequency modulation

Pulsing of a nano-ESI using relatively low-voltage modulation of a downstream electrode is noteworthy, considering that the nano-ESI is biased to a constant high potential sufficient for

continuous operation. Therefore, to further gain mechanistic insights into this pulsing, we investigated the relationship between the pulsed and continuous nano-ESI as summarized in Figure 6. Figure 6a illustrates that a constant potential applied to the nano-ESI over a wide range of voltages (1060–1560V) results in a continuous spray in the absence of gate modulation. The continuous ion flux is evident from a steady stream of ions with G2 permanently set to an open state (+50 V). However, for the same nano-ESI emitter biased to 1356 V, high-frequency modulation of the gate results in a pulsed nano-ESI behavior as shown in Figure 6b where a reverse dual-state operation is used to toggle the gate voltage between open (+50 V) and closed (–32 V) states. Lowering the G2 potential from +50 to –32 V enhances the electric field at the nano-ESI tip, however, this 82-V change is well within the voltage range for continuous operation of nano-ESI as shown in Figure 6a. Thus, pulsation of nano-ESI with G2 toggling must be a result of the rapid change in the electric field while the magnitude of the change is within the continuous operation regime.

We postulate that a sudden increase in the electric field at the tip of a continuously spraying nano-ESI would result in an enhanced spray and a burst of ions. However, it appears that nano-ESI does not recover rapidly enough from this momentary burst, and ion generation is ceased for a short period of time following the momentarily enhanced spray. Importantly, the emitter reverts to continuous spray if sufficient time is provided. This is shown in the inset of Figure 6b, zooming into pulse periods > 1000 μ s. A gradual increase in the baseline for all ions starting at 1900 μ s denotes a transition to a continuous nano-ESI where ions are generated in between the bursts as well. Remarkably, the ion bursts are induced reproducibly at high frequencies ($T < 2$ ms, $f > 500$ Hz). For example, in Figure 6b, T_0 values for ions are reproduced with standard deviations ranging from 2–3.7 μ s (<1% RSD), and T_0 peak heights are reproduced with 5–8.4% RSD based on triplicate scans acquired over 44 minutes.

The observations above suggest that G2 gate modulation induces a pulsation in the nano-ESI by perturbing the continuous spray via a momentary enhancement in electric field, causing a spray burst with ensuing spray cessation for a short period of time in between the bursts. We refer to this operating mode as perturbation-induced pulsing because allowing the nano-ESI to experience any of the gate voltages for a sufficient period of time (e.g., $T > 1.9$ ms in Figure 6) would lead to the development of a continuous spray. Consequently, this mode of operation is only observed at high modulation frequencies.

Impact of spray voltage on perturbation-induced pulsing of nano-ESI

The bias potential applied to the emitter affects the relative magnitude of the field perturbation at the tip. Thus, we investigated this parameter in our next set of experiments to understand its effects on pulsing and ion formation efficiencies. These experiments utilized a multi-analyte aqueous solution (3 μ M [TEtA]⁺, 2 μ M [TButA]⁺, 3 μ M [THepA]⁺, 25 μ M acetaminophen, 15 μ M arginine, 30 μ M hydrocortisone, 50 μ M sucralose) containing 0.1% formic acid as well as 200 μ M sodium chloride to promote sodiation. These analytes represent varying degrees of ionization efficiencies and charging agents, providing insight into ionization processes.

To determine the lowest voltage for perturbation-induced pulsing, a tri-state G2 pulsing scheme (see Figure 7 caption for details) was used with a period corresponding to the arrival

of [TButA]⁺. The spray voltage was then gradually increased until ions were detected. To ensure the stability of the spray, the minimum voltage was set to 5 V above the ion appearance threshold. Figure 7 illustrates the impact of 5 nano-ESI voltages (starting from the minimum voltage) on arrival curves of analyte ions including [TEtA]⁺, [TButA]⁺, [THepA]⁺, sodiated acetaminophen ([Act+Na]⁺), protonated arginine ([Arg+H]⁺), protonated acetaminophen ([Act+H]⁺), protonated hydrocortisone ([Crt+H]⁺), and sodiated sucralose ([Suc+Na]⁺).

Perturbation-induced pulsing is evident for the three lowest voltages in Figure 7 based on the distinct peaks within the T scan window. Notably, the arrival times of ions are minimally affected by the emitter voltage for all ions. However, the changes in peak intensities have a strong analyte dependence, denoting a variation in ionization conditions as discussed below.

[TEtA]⁺, [TButA]⁺, [THepA]⁺ are charged in solution and are surface active, representing easily-ionized analytes. The ion intensities for this group of analytes had minimal impact from spray voltage. Arginine is charged in solution, but it is not expected to be surface active because of its solvation by water. The ion intensity for this analyte increased by a factor of three upon increasing the voltage in pulsed mode. On the other hand, acetaminophen, hydrocortisone and sucralose are uncharged in solution. Hydrocortisone is expected to be surface active while acetaminophen and sucralose are well-solvated analytes in water. Notably, [Act+H]⁺, [Crt+H]⁺, and [Suc+Na]⁺ had high intensity enhancements of 34, 13, and 5 times, respectively, between the lowest and highest pulsing voltages. The drastic improvement of ionization for uncharged analytes indicates an enhancement of excess charge in the droplets available for ionization at higher voltages. Furthermore, the larger extents of enhancement for [Act+H]⁺ and [Crt+H]⁺ relative to [Suc+Na]⁺ suggests that protonation is improved more than that for sodiation.

The trends above imply formation of smaller droplets with larger charge-to-diameter ratios at higher voltages.^{29,30} Thus, the ionization is generally improved. However, smaller initial droplets lead to fewer Rayleigh fission cycles and less pre-concentration prior to ion formation.² As a result, the sodium concentration is reduced in ion forming droplets, which would explain the lower impact of voltage on sodiated ions compared to protonated ions. Acetaminophen presents an interesting case because both of its forms (sodiated and protonated) are detected. The protonated form is enhanced tremendously, while the intensity of the sodiated form does not change appreciably across the voltage range for pulsed operation, implying a competition between protonation and sodiation pathways that is shifted toward protonation at higher voltages.

A dramatic change in spray operation was observed when the nano-ESI voltage was increased to +1350 V (green trace) and +1375 V (orange trace) as shown in Figure 7. At these voltages, ion intensity followed a 1/T dependence, indicating continuous operation of the nano-ESI. The transition to continuous spray is further verified in Figure S2 by inspection of mass spectra at various pulse periods. Notably, similar mass spectra were observed at +1375 V regardless of pulse period (no ion selection), while mass spectra changed drastically with T using +1325 V, denoting selective ion transmission because of pulsed spray at lower voltages. Interestingly, Figure 7 shows significant ‘dips’ in the ion

intensity for all ions at +1350 V, while ‘dips’ were only observed for [Crt+H]⁺, [TButA]⁺, and [THepA]⁺ at +1375 V. The dips signify a momentary reduction in ion formation, possibly by the reduction in the electric field at the rising edge of the G2 voltage pulse. However, this perturbation is not significant enough to completely stop the spray between pulses. The observed arrival curves resemble those in inverse ion mobility spectrometry where a continuous ion flux is disrupted by the gates, creating dips in ion intensity traces.³¹ Importantly, the analyte-dependent appearance of ‘dips’ at +1375 V suggests differential ionization for the analytes. One possible explanation is the formation of multi-jets at higher voltages,²⁹ and a differential response of the jets to the field perturbation. Analyte dependence in this scenario would imply a fractionation of analytes between the jets. It is of note that the analytes displaying dips at the highest voltage belong to the highly surface-active group of analytes, supporting the potential role of analyte fractionation in the observed intensity dips.

Overall, the investigation of nano-ESI voltage indicates that the pulsed regime is achieved within a relatively narrow range of voltages applied to the emitter tip. This range depends on the size of the nano-ESI emitter tip and should be optimized for each emitter. Moreover, enhancement of low-ionization efficiency analytes is observed with increasing voltage. Thus, the highest voltage allowing pulsation is recommended for the best analytical performance of the pulsating nano-ESI. The sections below further explore the ionization process and associated advantages in perturbation-induced pulsed nano-ESI with coupled ion selection by comparing ion fluxes to those from a continuous nano-ESI at several emitter distances from the ion gate.

Ion flux in pulsed spray operation relative to continuous operation

The comparisons between pulsed and continuous nano-ESI modes were conducted using the same emitter tip at three tip-to-gate distances. For the pulsed mode, a tri-state operation with a 50-μs gate open duration was used (see Figure S3a for the pulse scheme), and the nano-ESI voltage was gradually increased at each distance to obtain the maximum intensity at T_0 for the ion with the longest T_0 . This voltage optimization procedure was devised to ensure that perturbation-induced pulsing was in effect for all values in the T scan window. Note that pulsed operation is compromised at larger T values by reversion to continuous nano-ESI as discussed above. This transition occurs faster at higher nano-ESI voltages, necessitating the use of late-arriving ions for voltage optimization. For the continuous spray mode, the ion gate was operated in an open state by setting G2 to +50 V.

Figure 8a compares the ion intensities in pulsed mode at the fundamental frequency of each ion (observed at T_0) to the intensities in continuous nano-ESI at a tip-to-gate distance of 5.4 mm. The pulsed mode was operated at an optimum emitter voltage of 1600 V, while a wide range of voltages were used in continuous nano-ESI to provide a broader comparison of the ion flux between the two modes. The ion intensity versus voltage curves for the continuous mode are provided in Figures S3b–S3i.

Importantly, Figure 8a shows that for most analytes, intensities in pulsed mode reach > 50% of those in continuous mode, denoted by ion intensity ratios > 0.5. These high ion intensity ratios are particularly noteworthy because the pulsed mode incorporates mobility-based ion

selection, while no ion selection is in effect for the continuous mode. The intensity ratios for hydrocortisone vary in a broader range (0.36–0.9) compared to the other analytes because the ionization efficiency of this analyte changes markedly in continuous mode as a function of emitter voltage (see Figure S3h).

To decipher the significance of the ion intensity ratios in Figure 8a, we compared these experimental values to expected values in a scenario where the nano-ESI is unaffected by gate modulation. For each analyte ion with a specific T_0 in the pulsed mode, the gate is open for only 50 μ s. Thus, if nano-ESI is unaffected by gate modulation, the continuous beam of ions for each analyte would be sampled with a duty cycle of 50/ T_0 in the pulsed mode. In other words, we expect ion intensity ratios equal to 50/ T_0 if pulsed and continuous nano-ESI produce similar analyte ion densities in their respective ion plumes. For the experiments in Figure 8a, T_0 = 426–749 μ s were used, leading to expected ion intensity ratios of 0.07–0.11. The significantly larger experimental ratios in Figure 8a indicate that the packets of ions generated in the pulsed mode are more concentrated (over 5 fold) in analyte ions compared to any segment of the ion beam generated by the continuous nano-ESI.

These experimental observations along with those in Figure 2 suggest accumulation of analytes at the tip in between spray pulses. Solution accumulation results from continuation of the gravity-driven flow in between spray pulses, while solvent evaporation may further concentrate the solution at the tip. If ionization efficiency is not altered by analyte accumulation at the tip relative to continuous nano-ESI, and 100% of the ion packet is sampled by the ion gate in the pulsed mode, one would acquire a ratio of 1.0 for all ions in Figure 8a. Notably, the ion arrival peaks have FWHM=44–64 μ s (Figure S4) at the tip-to-gate distance of 5.4 mm while the gate pulse width of 50 μ s is used. Therefore, part of the deviation from the value of 1.0 in Figure 8a originates from incomplete sampling of the ions. Thus, the > 0.5 ratios in Figure 8a imply that the ionization efficiency in pulsed nano-ESI may only be slightly reduced compared to that of the continuous mode.

As noted above, analyte accumulation between pulses may originate from solution accumulation at the tip. If there is significant solvent evaporation during this time, analyte preconcentration would also occur, potentially causing complications in ionization such as aggregation of analytes. To investigate the effects of solvent evaporation, we examined aggregation of acetaminophen by quantifying the protonated dimer to monomer ratio in pulsed and continuous modes under the experimental conditions of Figure 8a. Results are summarized in Figure S5, and show that the pulsed mode yielded a dimer:monomer ratio of 0.091 ± 0.001 ($\bar{x} \pm$ s.d., $n=3$), while values of 0.069–0.081 were obtained for the continuous mode depending on the nano-ESI voltage; the ratio of 0.069 corresponds to the optimal voltage (1900 V) for the intensity of protonated acetaminophen monomer in the continuous mode. Despite the five-fold higher analyte ion density in the pulsed plume, the dimer:monomer ratio is only marginally higher in this mode compared to that of the continuous mode. Thus, the results cumulatively suggest that the effect of solvent evaporation is minimal and the high intensity ratios in Figure 8a likely arise from spray of the accumulated solution at the tip with ionization efficiencies close to that of the continuous mode.

Effect of tip-to-gate distance on efficient perturbation-induced pulsed ionization

Figure 8b shows the intensity ratios between the pulsed and continuous modes using a tip-to-gate distance of 7.9 mm. The pulsed mode at this distance is operated at 1653 V, and the ion intensity ratios reach 0.3–0.7 for most ions (> 0.2 for hydrocortisone). These values are again drastically higher than expected ion intensity ratios (0.036–0.07 based on $T_0 = 739$ – $1373 \mu\text{s}$ and gate open time = $50 \mu\text{s}$), consistent with the analyte accumulation scenario discussed above. However, the intensity ratios at 7.9 mm (Figure 8b) are generally lower than those at 5.4 mm (Figure 8a). We attribute this effect largely to the optimization of the pulsed spray voltage. At a farther tip-to-gate distance, longer times are needed for ions to traverse this region, leading to increases in the time between pulses. This increase in time is particularly large for late-arriving ions. To ensure that the spray does not revert to continuous mode in between pulses, a lower emitter voltage is necessary, reducing the ionization efficiency. Notably, the peak widths observed at 5.4 mm and 7.9 mm distances are similar (see Figure S4), implying that ion packet sampling efficiency is not a major contributor to the lower intensity ratios in Figure 8b relative to Figure 8a.

At a tip-to-gate distance of 11.5 mm, the intensity ratios between pulsed and continuous nano-ESI are further reduced (0.06–0.5) as shown in Figure 8c, although they are still significantly higher than expected (0.02–0.038, $T_0 = 1303$ – $2494 \mu\text{s}$). We note again that the pulsed-nano-ESI voltage was limited by the long pulse periods needed at a farther distance, reducing ionization efficiencies. Another contributing factor to lower ratios in Figure 8c relative to Figures 8a and 8b is the peak widths. The wider peaks at 11.5 mm (see Figure S4) reduce the fraction of the ion packet sampled by the gate.

The effect of solution accumulation is also more pronounced at 11.5 mm because of longer times between spray pulses. Figure S6 illustrates this effect by examining the peak intensity ratios for T_0 and T_1 peaks of analytes. The ratios stay approximately constant (similar to Figure 2) for all analytes at distances of 5.4 and 7.9 mm, indicating that the increasing accumulated solution volume has a minimal impact on ionization efficiency within the wait times between spray pulses at these distances ($< 1400 \mu\text{s}$). However, at the tip-to-gate distance of 11.5 mm, a continuous reduction in $T_0:T_1$ intensity ratios is observed with increasing T_0 . This trend denotes a significant deterioration in ionization efficiency with T_0 , likely as a result of extensive analyte/solvent accumulation in between pulses at longer pulse periods.

Overall, these experiments show that larger tip-to-gate distances limit the upper value of the emitter voltage for perturbation-induced pulsing. Moreover, the longer T_0 s lead to extended solution accumulation. These factors generally reduce ionization efficiency in perturbation induced pulsing. Thus, the flux of mobility-selected ions become closer to those produced via continuous nano-ESI (no ion selection) at smaller distances. The tip-to-gate distance also impacts ion selection capabilities as discussed below.

Ion selection performance in perturbation-induced nano-ESI pulsing by an ion gate

Ion selection capabilities can be quantified using resolving power as defined by eqn. 2:

$$\text{Resolving Power} = \frac{T_0}{FWHM} \quad (\text{eq. 2})$$

where T_0 is the period for the fundamental peak and FWHM is the peak width at half maximum. Figure 9 shows the effect of the tip-to-G1 distance on resolving power using the data obtained in experiments of Figure 8. As the distance is increased, resolving power improves for all ions. This trend indicates a significant contribution from non-diffusional processes to the peak width. In other words, increasing the distance leads to longer travel times, but the ion packet widths do not grow proportionally (see Figure S4 for peak widths), resulting in improved resolving power. The peak widths have contributions from the spray duration, gate operation, and mobility-field inhomogeneity from the ESI field penetrating into the mobility area.

These data suggest that for the best resolving power, the tip should be placed farthest from the gate. However, ion flux from the pulsed nano-ESI decreases with distance as discussed above, creating a trade-off between ion flux and ion selection ability.

Suitability of the pulsed-nano-ESI with ion selection as a reagent ion supplier

For an initial evaluation of the suitability of pulsed-nano-ESI with ion selection as a general-purpose reagent ion supplier, we investigated the stability of ion selection, stability of ion intensities, the effect of solvent, and operation in negative polarity. The stability of ion selection and stability of ion intensities were evaluated by monitoring the variation in T_0 s and peak heights, respectively, for the 8 ions reported in Figure 8 over the duration of each data collection. The data at 11.5 mm were not considered because of compromised ionization efficiency as discussed above.

At a tip-to-G1 distance of 5.4 mm, triplicate scans were collected over 15.1 minutes, and the T_0 s were reproducible with standard deviations of 1.4–2.7 μ s (RSD: 0.25–0.60%), while the average RSD for peak heights was 4.2% (range: 2.1–7.1%) for the 8 ions. At the tip-to-G1 distance of 7.9 mm, triplicate scans were collected over a duration of 22.2 minutes with T_0 RSDs between 0.05–0.26% (standard deviations of 0.6–2.2 μ s), and the average peak height RSD was 13.3% (range: 8.8–15.3%). These data indicate reasonable reproducibility for pulsed nano-ESI and ion selection.

For the effect of solvent, we tested a 1:1 mixture of water:methanol with 0.1% formic acid as a common solvent for ESI using the same analyte concentrations as in Figure 8. 250 μ M of NaCl was also added to promote sodiation. A stable pulsation of the nano-ESI was not observed with this solvent using the 5- μ m emitter and operating conditions as those employed for aqueous samples. However, a stable perturbation-induced pulsed nano-ESI was obtained (see Figure S7) using an emitter with a smaller tip (approx. 2–3 μ m i.d.), a reduced counterflow gas rate (1 LPM), and an increased sample reservoir height. The effects from these factors indicate that the solvent accumulation rate plays an important role for stable operation in perturbation-induced pulsing, and should be carefully considered for each solvent composition. Additionally, the stability of the pulsed nano-ESI with 1:1 water:methanol solvent was evaluated as previously described. For triplicate measurements

over 30.3 minutes, the precision of T_0 s across the 8 ions was marked by a standard deviation of 0.3–3.2 μ s (T_0 RSDs: 0.03–0.26%), while the average peak height RSD was 9.6% (range: 1.5–18.4%).

Lastly, nano-ESI perturbation-induced pulsation in negative mode was tested using a 5- μ m emitter and an aqueous solvent as shown by the ion arrival curves of three chloride adducts in Figure S8 (see Figure caption for experimental details). Based on triplicate scans collected over 27.7 mins, T_0 s were reproducible with a standard deviation of 0.8–1.4 μ s (T_0 RSDs: 0.07–0.13%) and an average peak height RSD of 9.2% (range: 8.2–10.9%).

Collectively, the excellent precision of ion arrival times (T_0) and reasonable precision of ion flux (T_0 peak heights) across a diverse group of ions, under four experimental conditions suggest that this method of pulsed nano-ESI with coupled ion selection could be employed to deliver reagent ions at atmospheric pressure with rapid switching ability between ions based on their mobilities.

Conclusions

The experiments presented above indicate that a modest change in electric field may be used to pulse nano-ESI at high frequencies via reproducible perturbation of the continuous spray. When the electric field modulations are induced by an ion gate downstream of the emitter tip, ion generation can be synchronized with mobility-based separations, providing a facile means to generate and select ions at atmospheric pressure. Importantly, the ion flux from the pulsed nano-ESI operated in this regime is independent of the frequency of operation, suggesting accumulation of analyte solution in between spray pulses and minimal impact of accumulation extent on ionization efficiency. For aqueous solutions, only a marginal increase in analyte aggregation is observed in pulsed mode (analyte accumulation) compared to continuous mode (no analyte accumulation), suggesting that analyte accumulation is likely a consequence of solution accumulation at the tip with minimal solvent evaporation. The minimal impact of solution accumulation on ion flux is intriguing. For continuous nano-ESI, ion utilization efficiency (ion intensities normalized to solution flow rate) continually decreases with higher flow rates.^{32,33} If spray of the accumulated solution occurs within a constant time in pulsed spray, an analogous reduction in ion intensities is expected with increasing accumulated volume. Absence of this trend implies that the actual spray time per pulse increases with more solution accumulation at the emitter tip, maintaining a relatively constant effective flow rate for the pulsed spray. Such changes in spray duration would not be noticeable in our experiments if the spray durations are significantly shorter than the gate widths used. Further experiments would be needed to shed light onto these effects in future reports.

The efficient ionization of the accumulated analyte allows ion selection in atmospheric pressure with ion fluxes reaching >50% of those obtained with continuous nano-ESI where no ion selection is in effect. Remarkably, these attributes are obtained at nano-ESI voltages far lower than those used in continuous nano-ESI, prolonging the lifetime of the emitter tips.

The analyte accumulation mechanism also has implications for successful operation of pulsed nano-ESI with ion selection in various configurations. For example, the larger emitter-to-gate distance enhances the ion selection resolution. However, it requires a lower operating voltage to minimize the likelihood of spray reversion to continuous operation in between longer pulse periods, in turn reducing ionization efficiency. Excessive solvent accumulation at larger separation distances eventually leads to compromised ionization at low frequencies. Accordingly, ion fluxes for pulsed nano-ESI with ion selection become closer to those of continuous nano-ESI at shorter tip-to gate distances. In general, a careful selection of experimental parameters should be considered to maintain optimal solvent accumulation for stable pulsing. These parameters include the solvent delivery rate to the tip, the solvent evaporation rate at the tip which is affected by gas flows and solvent type (e.g. organic versus aqueous), emitter tip dimensions, tip surface wettability, and the voltage applied to the emitter.

Overall, the ease of assembly and operation as well as reasonable ion selection capabilities render the reported technique an attractive approach for supplying mobility-selected reagent ions for atmospheric pressure reactions such as chemical ionization. Solution accumulation (droplet formation at the tip) in pulsed mode and comparable sensitivities relative to continuous nano-ESI also underscore encouraging avenues for enhancing ionization. For example, sequential ejection of ions from spraying droplets may be explored, effectively incorporating an in-situ separation of analytes within the droplets based on affinity to air-water interface as demonstrated in probe electrospray ionization,³⁴ which may reduce matrix effects in ionization.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Schematic of nano-ESI source placed in a PCB ion guide housing with resistively coupled electrodes and a three-grid ion shutter interfaced to MS. Note that the schematic is not drawn to scale, and the MS ion sampling voltages represent positive mode analysis.

Figure 2.

Normalized ion intensities as a function of pulse period (T) using constant $100 \mu\text{s}$ pulse width (Figure inset). Intensities for each ion are normalized to the maximum ion intensity observed during the T scan. Data were generated by scanning T at $10 \mu\text{s}$ increments with the following experimental parameters: $E1 = +600 \text{ V}$, $G1 = +100 \text{ V}$, $G3 = 0 \text{ V}$, spray voltage = $+1339 \text{ V}$, tip-to- $G1$ distance = 8.7 mm , aqueous solution of $[\text{TEtA}]^+$, $[\text{TButA}]^+$, $[\text{THepA}]^+$.

Figure 3. Relationship between the timings of peaks observed for each analyte in Figure 2 indicate harmonics defined by eqn. 1.

Figure 4.

Simulated ion density and transmission at the ion gate (a and c), and MS intensities (b and d) for continuous (a, b) and pulsed nano-ESI (c, d) operation. In a and c, ion transmission efficiency through the ion gate is shown with the red trace, ion density at the gate is shown by the blue dashed line, and the ion detection trace (overlap of ion density at the gate and ion transmission efficiency) is shown by the green dashed line. (a) Continuous nano-ESI with $T = 500 \mu\text{s}$ (2 kHz) pulsing of G2 and gate pulse width of 100 μs . (b) Detected ion intensity by MS from a continuous nano-ESI as a function of gate pulse period (T) scanned with 5 μs increments for T. (c) Pulsed nano-ESI operating in sync with G2 (shown at $T = 500 \mu\text{s}$). Discrete ion packets are generated from each G2 pulse, and shifted in time by 1080 μs (time for an ion to traverse tip-to-gate distance). Each ion packet at the gate is generated by a 1080- μs earlier gate pulse as illustrated by the black brackets. (d) Detected ion intensity by the MS for an ion with tip-to-gate travel time of 1080 μs simulated by scanning pulse period (T) with 5 μs increments.

Figure 5.

(a) Ion arrival curves (detected intensities as a function of T) for $[\text{TButA}]^+$ using dual-state (black trace) and reverse dual-state (orange trace) operation of G2 (see inset for voltages) with a constant 100 μs gate pulse width. Zoomed-in areas around T_0 comparing the two pulse modes at 100 μs (solid lines) and 150 μs (dotted lines) gate pulse widths are shown for (b) $[\text{TEtA}]^+$, (c) $[\text{TButA}]^+$, and (d) $[\text{THepA}]^+$. Data were generated using an aqueous solution of the three analytes by scanning T at 10 μs increments with the following experimental parameters: $E1=+600\text{V}$, $G1=+100\text{V}$, $G3=0\text{V}$, spray voltage = +1339 V, and tip-to-G1 distance = 8.7 mm.

Figure 6.

(a) Extracted ion intensity for three TAA ions using continuous operation of nano-ESI by setting G2 = +50 V. Data were generated with 30-s acquisitions, increasing the spray voltage at 100 V increments and using 90 s stabilization time between acquisitions. (b) Ion intensities as a function of T using the same emitter with electrospray voltage of 1356 V and reverse dual-state pulse applied to G2 with gate pulse width of 100 μs (see Figure 5a inset for pulse shape). T was scanned with 10 μs increments. Experimental parameters in both modes: E1 = +600 V, G1 = +100 V, G3 = 0 V, tip-to-G1 distance = 6.2 mm.

Figure 7.

Effect of spray voltage on ion intensities for (a) [TEtA]⁺, (b) [TButA]⁺, (c) [THepA]⁺, (d) [Act+Na]⁺, (e) [Arg+H]⁺, (f) [Act+H]⁺, (g) [Crt+H]⁺, (h) [Suc+Na]⁺. T was scanned at 10 μs increments, using a tri-state pulse supplied to G2 with the following parameters: ion blocking voltage = -25 V, ion transmit voltage = +42 V (50 μs), ion push voltage = +100 V (30 μs). Additional experimental parameters: E1 = +500 V, G1 = +83 V, G3 = 0 V, tip-to-G1 distance = 6.5 mm, N₂ counter flow gas (entering MS) = 3 LPM.

Figure 8.

Ratios of ion intensities at T_0 in pulsed mode with optimum nano-ESI voltage to those from continuous nano-ESI operated at a range of voltages. Pulsed nano-ESI was operated using the tri-state pulse scheme detailed in Figure S3a and the following experimental parameters: $E1 = +600$ V, $G1 = +100$ V, $G3 = 0$ V. Tip-to-G1 distance and pulsed nano-ESI voltage: (a) 5.4 mm and 1600 V, (b) 7.9 mm and 1653 V, (c) 11.5 mm and 1715 V. Note that ions in the legend are arranged in order of decreasing gas-phase mobility (top to bottom). Error bars represent standard deviations of ratios calculated from triplicate scans in pulsed mode and standard deviation of ion intensities within 30-second acquisitions in continuous mode.

Figure 9.

Resolving power (equation 2) determined at three tip-to-grid distances with ions arranged in order of decreasing gas-phase mobility. Data were generated using same experimental parameters detailed in Figure 8 with the tri-state pulse detailed in Figure S3a. [Arg+H]⁺ is excluded from the list of analytes compared to Figure 8 due to the presence of unresolved peaks.