High-Throughput Mass Spectrometry

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Induced Nanoelectrospray Ionization for Matrix-Tolerant and High-Throughput Mass Spectrometry**

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Electrospray ionization (ESI) mass spectrometry is used extensively in biomolecular analysis, including proteomics, metabolomics, and glycomics. The newer nanoelectrospray method (nESI, flow rate $< 1000\ nL\ min^{-1}$)[1–6] is derived from ESI, but uses a small-diameter spray tip that leads to improved ionization efficiency. Advantages of nESI over conventional ESI include 1) much improved ionization efficiency and ion transmission, 2) greatly reduced ion suppression and matrix effects, 3) longer analysis times that facilitate identification by tandem mass spectrometry (MS/MS), and 4) smaller sample volumes and lower absolute sample amounts, which improve compatibility with high-efficiency separation techniques, including capillary electrophoresis.[7] In spite of these advantages, there is a growing need for still higher sensitivity and throughput, and greater ease of operation than is provided by current nESI methods. In previous work, we have demonstrated that accurate control of the spray time and its synchronization with the inlet of a desorption electrospray ionization (DESI) mass spectrometer improves sample utilization efficiency by over 100 times.[8] Herein we describe an induced nESI method that provides new capabilities for ESI and nESI. The method is characterized by remarkable tolerance to matrix effects and high ionization efficiency. Unique features are: 1) human urine and serum samples can be analyzed directly, 2) physical electrical connections are avoided, thus facilitating operation of arrays of nESI emitters, 3) high sensitivity and better sample economy are achieved, as indicated by the fact that 1.5×10^{-9} L peptide solution ($1\ \mu g\ mL^{-1}$) provides 45 min of spectral acquisition with a signal-to-noise ratio greater than 50 by using a commercial benchtop mass spectrometer, and 4) both positive- and negative-ion spectra can be recorded during one spray cycle.

The methods used to make electrical contacts to ESI emitters include simply inserting a wire conductor into the capillary,[9,10] applying the voltage to an electrode in contact with the solution upstream of the emitter,[11,12] coating the emitter with conductive material,[1,2,13] and use of a dielectric barrier, which avoids physical contact but operates at 0.5–2 Hz.[14,15] However, none of these methods is completely adequate, especially for operating nESI arrays.[16–18] The induced nESI method reported herein facilitates high-throughput measurements and avoids physical contact between electrode and spray solvent. Figure 1 shows an nESI array in which eight nESI emitters loaded with different samples were mounted to a fixed pulley driven by a moving belt. The electrospray potential (2–4 kV) is applied to a single electrode that is appropriately placed so that each of the spray emitters approaches to within 2 mm of it in turn. The applied potential is pulsed repeatedly in the positive mode at a frequency that can be anywhere in the range 10–2000 Hz, but is typically set at 50 Hz. Strong dynamic electromagnetic fields are produced in the adjacent nESI emitter to result in a burst of nESI droplets (Figure 1a). Figure 1b shows the sampling position and Figure 1c–f show mass spectra recorded for four of the emitters as they pass by the electrode

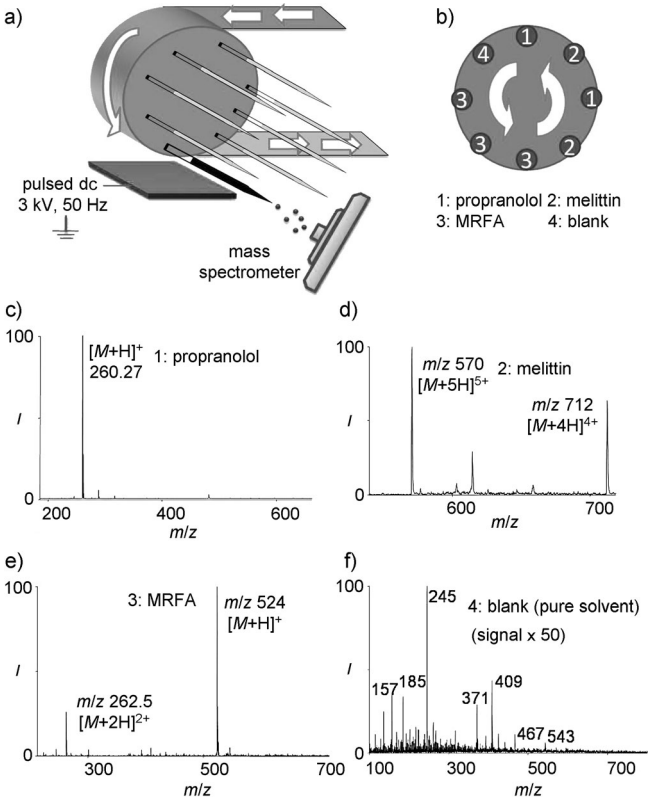

Figure 1. a) Diagram of high-throughput induced nESI array, b) sample loading sequence (1: propranolol ($1\ \mu g\ mL^{-1}$), 2: melittin ($5\ \mu g\ mL^{-1}$), 3: MRFA (Met-Arg-Phe-Ala; $2\ \mu g\ mL^{-1}$), and 4: blank (methanol/water 1:1)); single scan mass spectra of c) propranolol ($1\ \mu g\ mL^{-1}$), d) melittin ($5\ \mu g\ mL^{-1}$), e) MRFA ($2\ \mu g\ mL^{-1}$), and f) blank (methanol/water 1:1) Note the different vertical scales.

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in turn. The fixed pulley is driven at a speed of four samples per second (30 rpm) using preloaded samples (Video 1 in the Supporting information). It should be noted that null spectra recorded at emitter positions distant from the electrode show no analyte ions (Figure S1 in the Supporting information). The results were obtained with an ion trap mass spectrometer (LTQ, Thermo Finnigan) using an ion injection time of 50 ms (corresponding to ca. 2 RF pulses, 50 Hz) and an ion analysis time of approximately 70 ms, that is, a single mass scan was recorded each time an emitter passed by the electrode.

Both commercial and home-made nESI emitters were used (Figure 2a). The pulsed nature of the inductively formed spray is shown in Figure S2 in the Supporting Information. Figure 2b shows that as little as 1.5×10^{-9} L peptide (melittin) solution (1 μg mL^{-1}) is enough to provide a mass spectrum continuously for 45 min with a signal-to-noise ratio of approximately 50 by using a benchtop mass spectrometer. The times and scan rate used suggest that only 100 zeptomol

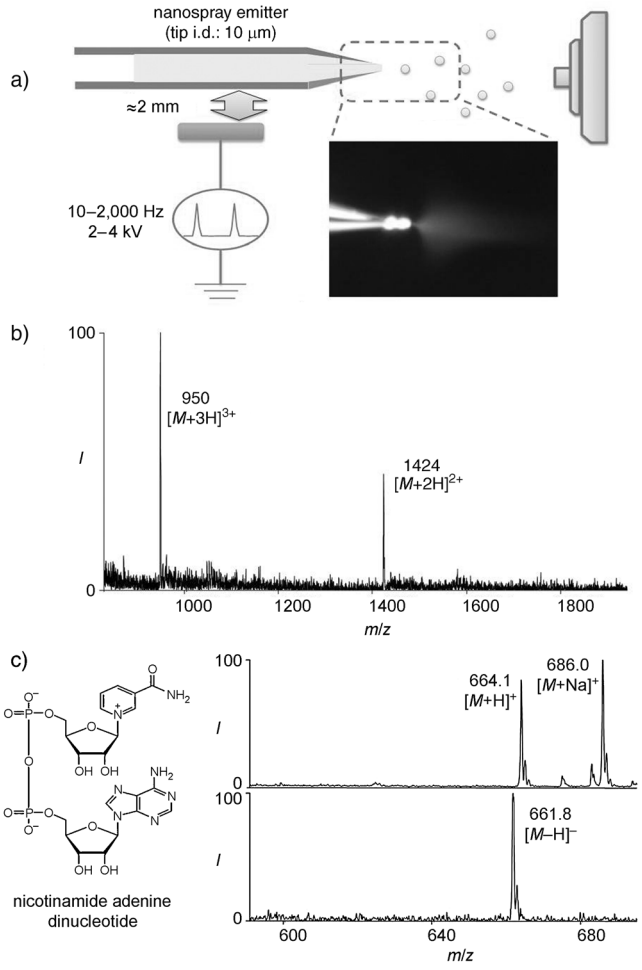

Figure 2. a) Induced nESI using1 μM analyte in 1:1 MeOH/H_2O. The emitters were pointed towards the mass spectrometer inlet (5 mm away) with the electrode 2 mm away from the emitter, but no physical contact. The image shows an induced nESI plume, applied voltage pulse train (50 Hz, 3.8 kV). b) Single-scan positive-mode mass spectrum obtained using 1 μg mL^{-1} melittin with 2 μm tip, 3.5 kV pulse voltage (10 Hz) taken from a continuous 45 min scan. Ion intensity: 3.92×10^2. c) Induced nESI MS of nicotinamide adenine dinucleotide (10 μM) in both positive- and negative- ion modes.

(1×10^{-19} mol) of sample is utilized in acquiring each mass spectrum. When using the same solution and emitter, conventional nESI gave spectra with similar signal-to-noise ratio, but only for 10 seconds (Figure S3 in the Supporting Information). It should be noted that the increase in efficiency observed here is not related to the 100-fold improvement observed in synchronized DESI.[8] In that study, increased performance was mainly the result of synchronizing the pulsed ionization source with opening of the discontinuous inlet to a miniature mass spectrometer. However, in the present study, improved ionization efficiency is attributed at least in part to the fact that induced nESI provides electrospray pulses at an overall average flow rate of around 30 pL min^{-1}. The overall spray flow rate is observed to depend on the frequency of the applied alternating current (ac); the lowest frequency that provides a stable spray was 10 Hz, which corresponds to a flow rate of 30 pL min^{-1}. It is well known that a lower spray flow rate results in a higher ionization efficiency,[19] presumably because of smaller initial droplet size, although this behavior was not confirmed by direct measurement. Compared with the well-established automated nESI chip technique using etched nozzles (ca. 0.4 samples per minute, spray rate ≈ 100 nL min^{-1}),[20,21] induced nESI provides stable spray ionization when using simple pulled spray emitters and with a somewhat higher throughput (4 samples per second).

It should also be noted that over the available ac frequency range (10–2000 Hz), both positive and negative ions are generated when continuously applying the pulsed potential (Figure 2c). Spectra of both polarities can be obtained simply by switching the polarity mode of the mass spectrometer, thus providing evidence for virtually simultaneous production of ions of both polarities from a single spray emitter, as also observed in inductive desorption electrospray ionization.[8] For example, for a solution of nicotinamide adenine dinucleotide (Figure 2c), mass spectra can be recorded in both modes to produce protonated and sodiated ions in the positive-ion mode and deprotonated molecular anions in the negative-ion mode. This capability allowed identification of two other peptides by recording tandem mass spectra of the multiply charged precursor ions $[M+4H]^{4+}$ and $[M+3H]^{3+}$ for melittin, and $[M-4H]^{4-}$, $[M-3H]^{3-}$, $[M-2H]^{2-}$, and $[M-H]^-$ for YEEI (Glu-Pro-Gln-Tyr-(PO_3H_2)-Glu-Glu-Ile-Pro-Ile-Tyr-Leu; Figure S4 in the Supporting Information).

The noncontact nature of induced nESI means that it can be implemented on nESI arrays of various geometries. A simple example uses three emitters oriented parallel to the axis of the mass spectrometer with a copper electrode placed 2 mm from the array (Figure 3a). Pulsed voltages were generated inductively and virtually simultaneously in all three emitters. When a 3 kV positive pulse (50 Hz) was applied to the electrode, simultaneous spray plumes were observed from all three emitters (expansion in Figure 3a). All four analytes were observed in the form of protonated propranolol, atenolol, and cocaine at m/z 260, 267, and 304, respectively, in the positive-ion mode (Figure 3c) and deprotonated 4-chlorobenzonic acid ions at m/z 155 and 157 in the negative-ion mode (Figure 3b).

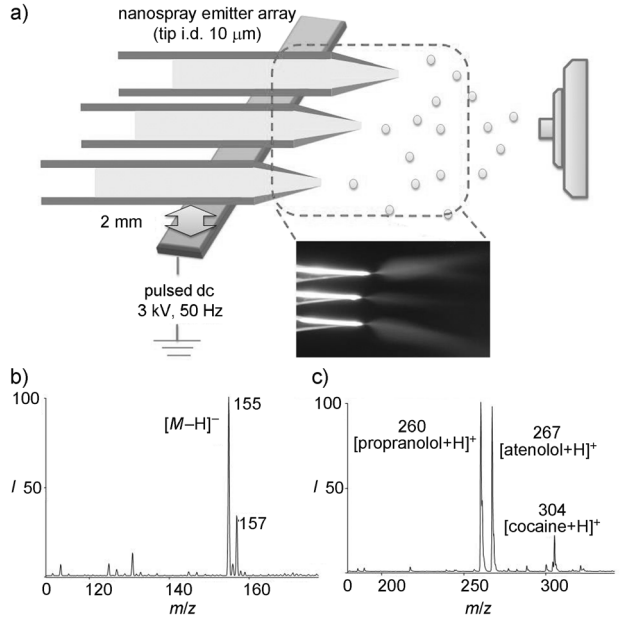

Figure 3. a) Induced nESI array. Three nESI emitters loaded with solutions (methanol and water 1:1) containing analytes: propranolol (500 ng mL^{-1}), 4-chlorobenzonic acid (100 ng mL^{-1}), and mixture of atenolol (500 ng mL^{-1}) and cocaine (50 ng mL^{-1}). nESI emitters were pointed at the mass spectrometer inlet (5 mm away) with the electrode 2 mm from the emitters. The image shows the induced nESI array plumes, taken at 50 Hz, 3 kV. b) Mass spectrum (negative-ion mode) of 4-chlorobenzonic acid. c) Mass spectrum (positive-ion mode) of propranolol, atenolol, and cocaine.

Importantly, induced nESI exhibits a remarkable tolerance to matrix effects, thus minimizing sample pretreatment. In this respect, induced nESI is similar to many ambient ionization techniques.[22–25] A striking result is that raw serum, which is difficult to analyze by conventional nESI, can be analyzed directly by induced nESI mass spectrometry without sample pretreatment (Figure S5 in the Supporting Information). Propranolol can be identified and quantified after being spiked into raw serum in the concentration range of 2–800 ng mL^{-1}. Another feature of induced nESI ionization is that it can be used to analyze high salt solutions, where clogging occurs with conventional nESI. Using a commercial nESI emitter (tip i.d. 2 μm) and methanol/water (1:1) spray solvent, melittin was examined at various concentrations (1–50 g L^{-1}) in sodium chloride solutions. At the highest concentration (50 g L^{-1}), induced nESI lasted for at least 5 min without clogging, and the melittin precursor ion could be observed together with sodium chloride clusters (Figure S6 in the Supporting Information). The stability of the induced nESI system is illustrated with (artificial) urine, which was continuously sprayed for over 50 min (Figure S7 and Table S1 in the Supporting Information). The remarkable resistance to deleterious effects of high salt concentrations is ascribed to the removal of crystallized salt from the emitter tip. The alternating induced voltage, which is responsible for the quasi-simultaneous production of both positive and negative ions, also causes the ion flow to change direction within the solution, thus alternately drawing ions of given charge towards and away from the tip and therefore setting up a unique self-cleaning effect. This explanation is supported by the observation of a back-flow on the outside of the nESI emitter when the solution is sprayed, and of salt accumulation on the outside of the tip (Figure S6 in the Supporting Information). As a result, much less clogging was observed in induced nESI, which minimizes the need for desalting pretreatment.

Various compounds, including amino acids, peptides, dinucleotides, proteins, and therapeutic drugs were analyzed by using induced nESI. Most spectra are similar to those recorded by conventional nESI (examples are given in Supporting Information, Figure S8). The concentrations of the analytes range from 0.5 ng mL^{-1} to 50 μg mL^{-1}, and linear dynamic ranges are typically two or three orders of magnitude.

As indicated by preliminary results, the mechanism of induced nESI is related to that of both conventional direct current (dc) nESI and ac ESI.[26] Similar internal energy distributions were found for inducted nESI and conventional nESI by using the survival yield method (Figure S9 in the Supporting Information). Induced nESI was, however, found not to follow the Maxwell–Wagner electric stress behavior at the drop tip, as observed in ac ESI, which is usually operated above 10 kHz.[26] Induced voltages needed to initiate the spray and achieve maximum signal are 1.0 kV and 1.3 kV (peak-to-peak values), which are similar to those for conventional nESI (Figure S10 in the Supporting Information). In addition, induced nESI allows direct manipulation the charge-state distribution (Figure S11 in the Supporting Information). This process usually requires changing the spray solvent.

In summary, induced nESI ionization is presented here as a novel ionization method derived from conventional nESI. In various biomolecular applications, this technique shows the advantages of 1) greatly facilitating nESI array operation and thus potentially increasing the throughput of nESI, 2) ultrahigh sensitivity and sample economy, 3) almost simultaneous generation of ions of both polarity, and 4) compatibility with raw serum, whole urine, and concentrated salt solutions.

Experimental Section

All experiments were carried out with an LTQ mass spectrometer (Thermo Scientific, San Jose, CA). Capillary temperature: 150 °C; capillary voltage: 15 V; tube lens voltage: 65 V. A home-built power supply provided a positive pulsed output of 10–2000 Hz and 0–8 kV. The spray solution was mixture solutions of MeOH/water (ratio varies by compound), pure water solution (added with different concentrations of salts), artificial urine, and raw serum. Commercial silica nanoelectrospray tips of 5–20 μm were obtained from New Objective (Woburn, MA, USA). Silica nanoelectrospray tips are 10 μm unless otherwise indicated. Solution samples were prepared by diluting a stock solution, which was directly loaded to spray emitter with pulled pipette tips. For the induced nESI array, eight parallel holes were drilled into a fixed aluminum pulley, each nESI emitter preloaded with sample was inserted into one of the holes and pointed at the mass spectrometer inlet. The pulley was driven by a moving belt with a tunable speed between 1–90 rpm. A metal electrode 1 × 5 ×

1.5 mm was placed near the emitters to induce nESI at a distance of closest approach of 2 mm to the emitters.

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