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On-Demand Mass Spectrometry Analysis by Miniature Mass Spectrometer

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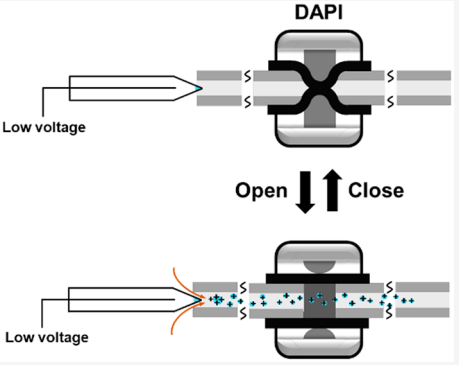
ABSTRACT: Electrospray ionization (ESI) has become a powerful tool for the analysis of biomolecules by mass spectrometry (MS). The process of ESI is difficult to control, and side reactions such as electrochemical reactions can occur during the ESI process because of the high voltages applied. Herein, a novel on-demand MS analysis method was developed based on discontinuous ion injection-induced ESI on a miniature MS system. Highly efficient ionization was enabled under low voltages (<300 V) using a discontinuous atmospheric pressure interface. On-demand ionization showed comparable sensitivity with regular nanoESI for the analyses of a series of compounds. It was found to be softer than regular ESI or nanoESI methods for ionization of proteins such as myoglobin and cytochrome C. As the ionization finished as soon as the interface was closed, the sample consumption was observed to reduce significantly for MS analysis, allowing single-cell analysis with multiple MS and MS/MS measurements.



INTRODUCTION

Mass spectrometry (MS) has become a powerful tool for proteomics, metabolomics, and other biochemical applications due to its unique capability of providing highly specific molecular information and performing qualitative analysis to complex samples. Electrospray (ESI)[1,2] is the most widely used ionization technique for biochemical applications due to its nature of softness, relatively high efficiency to most of biochemical compounds, and good compatibility with chromatographic separation. As a typical soft ionization technique, ESI is also useful for the investigation of structural, dynamic, and conformational properties of proteins by providing multiply charged protein ions directly from solution.[3]

To perform an ESI, high voltage (~3−5 kV) is typically required, where electrochemistry can occur with the electron transfer at the metal−liquid interface of the ESI emitter;[4] other reactions could also be induced with reactive oxygen species produced in the corona discharge.[5] This may cause problems in the characterization of protein structure information such as post-translational modifications. Using a lower flow rate, nanoESI is now more commonly used in proteomics or native MS.[6] However, a relatively high voltage (usually >1.2 kV) is still required to induce nanoESI, especially when a high percentage of water is used in the solution. Although ESI with relatively low voltages has been reported for studies using microchip-based devices,[7] decreasing the voltage of regular nanoESI or ESI to subkilovolts is still very challenging for MS analysis. Some voltage-free spray ionization methods have been developed,[8−10] such as droplet-assisted inlet ionization, solvent-assisted inlet ionization (SAII), and vibrating sharp-edge spray ionization. To maximize the efficiency of sample preparation, on-demand ionization strategies have been developed in some spray ionization MS approaches.[11−15]

In this work, we developed a novel method that can be used for ionization of compounds such as drugs and proteins at ESI voltages as low as 250 V without loss of sensitivity for MS analysis. The ionization was synchronized with the opening of a discontinuous atmospheric pressure interface (DAPI)[11] of a miniature mass spectrometer. On-demand ionization was realized, which significantly improved the utilization of samples in MS analysis, especially for the analysis of extremely small-volume samples such as singe cells.

RESULTS AND DISCUSSION

Development and Optimization of the on-Demand Ionization Method. The on-demand method was demonstrated on a modified miniature MS system (Figure 1a). It consisted of a dual-LIT (linear ion trap) mass analyzer,[16] a compact pumping system, a DAPI for ion injection, and a built-in source for nanoESI. To perform the on-demand nanoESI, the pulled tip of a glass capillary (1.5 mm o.d. and 0.86 mm i.d.) was slightly inserted into the inlet stainless-steel tubing of the DAPI (setup shown in Figure S1). At a low

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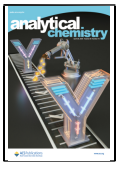

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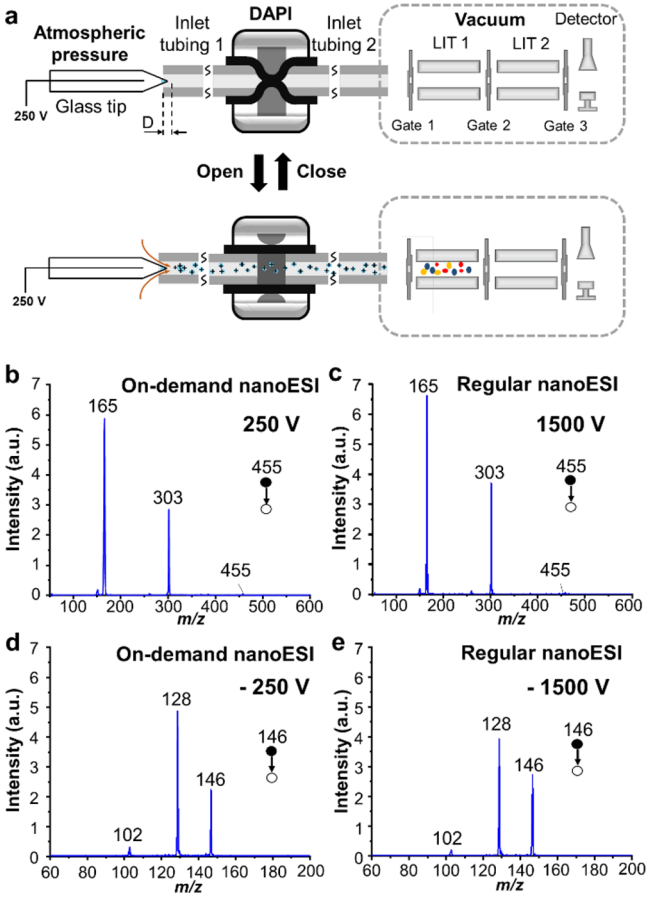

Figure 1. (a) Scheme of the DAPI-based nanoESI-MS system. Mass spectra of verapamil (200 ppb) by (b) on-demand nanoESI with voltage of 250 V and (c) regular nanoESI with voltage of 1500 V. Mass spectra of glutamate by (d) on-demand nanoESI with voltage of −250 V and (e) regular nanoESI with voltage of −1500 V.

voltage of 250 V, ionization was not induced when the DAPI was closed because the Rayleigh limit could not be reached, although a Taylor cone might be formed. When the DAPI was opened, it was interesting to observe that abundant ions were detected through the MS scan, indicating the occurrence of ionization. Tested with verapamil (200 ppb in methanol), on-demand nanoESI at an ionization voltage of 250 V (with the glass tip inserted at 1.7 mm) showed comparable sensitivity with regular nanoESI at an ionization voltage of 1500 V (Figure 1b and c, Figures S2 and S3). The on-demand ionization in both positive and negative ion modes can be readily implemented by switching the polarity of the spray voltage, for example, the analysis of glutamate (2 ppm) (Figure 1d and e) and PC 16:0/18:1 and peptide VLSPEYLWDDR (Figure S4a and b)

The effect of the voltage on the on-demand nanoESI was further investigated using verapamil in a methanol solution (200 ppb). Using a picoammeter connected between inlet tubing 1 and ground, the spray current on inlet tubing 1 was monitored. The spray ionization status was estimated by measuring the current curve. When a spray voltage of 230 V was applied, no spray current was observed. When the DAPI was opened for 20 ms, however, a pulse with a peak current of 6 nA was detected (Figure 2a). When a higher voltage of 300 V was applied, a constant spray current was detected even when the DAPI was closed. This result showed that regular

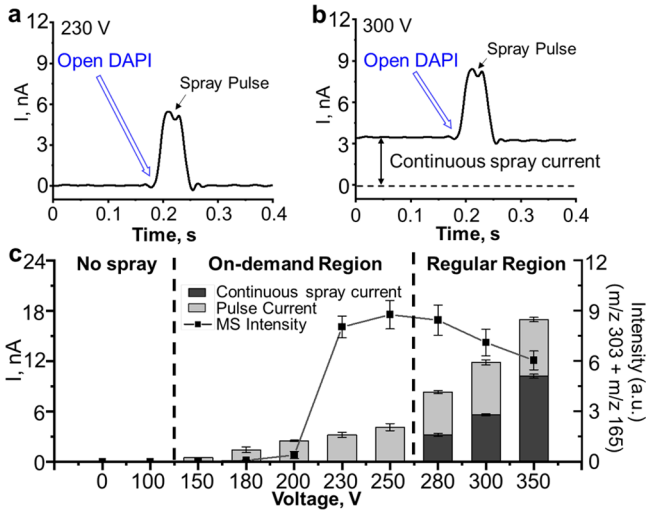

Figure 2. (a–b) Changes of spray currents under voltages of 230 and 300 V with opened and closed statuses of the DAPI. Tapered glass tips with o.d. of 3 μm were used, with an inserted depth of 1.7 mm. (c) Effect of voltages on the ionization efficiency, continuous spray current, and pulse spray current.

electrospray happened in the absence of gas flow when higher voltages were applied. When DAPI was operated, a current pulse could also be observed as an increase to the constant spray current (Figure 2b). It should be noted that voltages needed can still be affected by the solvent composition. With the increase of the volume ratios of water:methanol from 0% to 100% (0%, 50%, and 100%), the onset voltage of on-demand ionization was found to increase from 200 to 450 V (Figure S5). The correlation of the intensity of verapamil fragment ions with the spray status as a function of spray voltage was investigated (Figure 2c). Efficient ionization of verapamil was not observed when spray voltages lower than 200 V were applied. Good ion intensity for verapamil was observed at higher voltages, but the ionization became less stable when the voltages were higher than 400 V, which may be caused by discharging under higher voltages. It is worth noting that the trend of the verapamil ion intensity does not correlate well with the spray current measured. While the spray current increased constantly with the spray voltage, especially with the onset of the continuous spray current (4–9 nA) at voltages higher than 280 V (Figure S6), the verapamil ion intensity reached its maximum at 230–250 V before the voltage was high enough to induce the continuous spray. This further demonstrated that the ionization efficiency was not determined only by the spray but also by other steps in the entire process, such as the desolvation.[17,18] A hysteresis effect[19] was also observed when the voltage was changed stepwise, in which the spray can be maintained under voltages lower than 200 V (Figure S7). At voltages between 230 and 250 V, obviously the gas flow induced by the DAPI operation played a critical role for onsetting the spray but also provided an optimal condition for desolvation of the ions. Small droplets could be produced from the spray with the combination of the spray voltage and the gas flow. This optimal ionization process also synchronized with the ion introduction through DAPI, leading to an efficient on-demand use of the sample.

The effect of insertion depth of the tapered tip into the tubing inlet was also investigated, which was expected to affect the gas dynamics for the ionization significantly. As shown in

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Figure S8a−c, the highest ion intensity was observed at 1.8 mm and then decreased rapidly with further insertion of the tip into the inlet tubing. When tapered glass tips with larger sizes were used, a smaller depth was required to induce the ionization. However, the ion intensity was not related to the depth of the insertion when a fused silica capillary (i.d. 75 μm and o.d. 150 μm) was used (Figure S8d and Figure S9). Results from these experiments showed that the gas flow induced from the discontinuous injection was necessary to initiate the spray ionization at the voltages set. The gas flow speed, which varied with the insertion depth for the tapered tip, can also affect the ionization efficiency. This was verified with spray tips with different tip sizes and different taper angles (Figure S8 and Table S1).

The gas dynamic effect associated with the DAPI operation was further investigated with simulations (Figure S8e).With typical DAPI parameters set for the simulation, the gas flow speed was in the range of 280−380 m/s, which could induce an ionization process similar to a sonic spray.[20] It should be noted that the sonic spray or electrosonic spray ionization methods typically consume compressed gas at a high rate (1−10 L/min or higher), while the on-demand ionization method explored here did not require any high-pressure gas supply. This represents a significant advantage for applications using miniature mass spectrometers. The impact of the inlet opening area on the linear velocity/volume flow rate of the induced air flow during on-demand nanoESI is shown in Figure S8f, based on the simulations (see details in the Supporting Information). Similar with a sonic spray which was induced by high-speed gas flow,[21,22] a proper gas flow rate was required to obtained high efficiency by on-demand nanoESI.

The configuration of the on-demand ionization method in this work is to some respect similar to that of ESII[23,24] or captive spray ionization;[25] however, the ionization mechanism is significantly different. ESII is developed from SAII,[24] in which ionization is primarily based on the evaporation of solvents inside a capillary under high temperature and a gas flow induced by a pump. In ESII, application of a voltage improves the efficiency of SAII. In captive spray ionization, ionization is based on ESI, and the application of a gas flow sweeps around the emitter tip to help desolvation and to focus the spray into the MS inlet capillary. The on-demand ionization method in this work uses high-speed gas flow produced by the opening of the DAPI to induce sonic spray-like ionization, without heating of the inlet tubing or the solvent. Both electrospray and sonic spray are closely associated with the on-demand ionization method.

Ionization of Proteins. In previous studies using high velocity gas to facilitate ESI of proteins,[26,27] it was found that softer ionization could be achieved. Figure 3a shows the MS spectra of a myoglobin aqueous solution (with 0.03% acetic acid, *v*/*v*) acquired using the on-demand nanoESI. Abundant peaks with +9 and +10 charges were detected, while other peaks with higher charge states were at very low abundance. The charge distribution was obviously different with that obtained from regular nanoESI at 1800 V, in which the most abundant myoglobin peaks were centered on the peak with +15 charges (Figure 3b). A similar shift in charge distribution was also observed for the analysis of cytochrome C (aqueous solution with 0.03% acetic acid, *v*/*v*) by on-demand nanoESI (Figure 3c) in comparison with regular nanoESI (Figure 3d). These results showed that on-demand nanoESI is a softer ionization method for protein analysis. Analysis of protein by

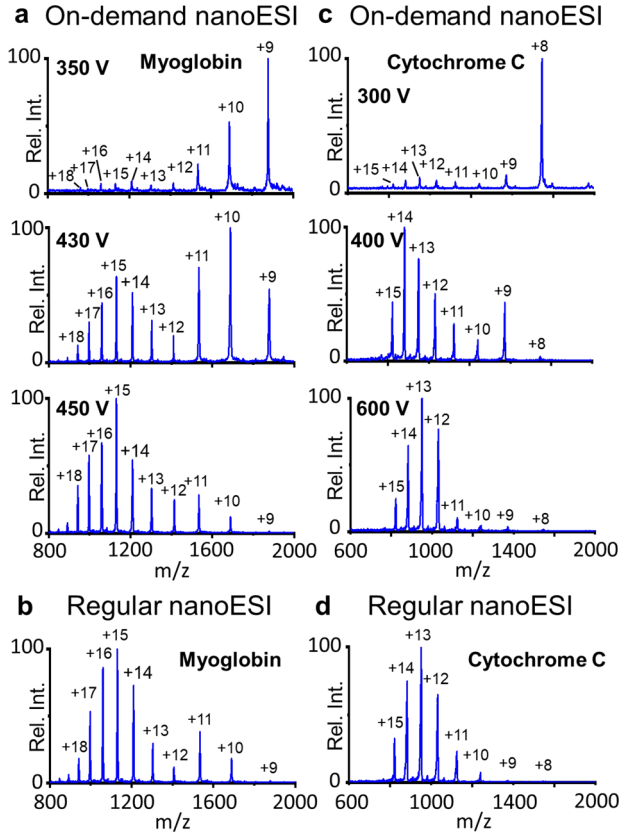

Figure 3. Mass spectra of myoglobin (200 ppm) by (a) on-demand nanoESI (350−450 V) and (b) regular nanoESI (1800 V). Mass spectra of cytochrome C (200 ppm) by (c) on-demand nanoESI (300−600 V) and (d) regular nanoESI (1800 V).

sonic spray ionization and electrosonic spray[26,27] required use of compressed gas, while here no additional gas supplied was needed. Although methods such as ESII and SAII[28] have also been demonstrated to be softer than ESI methods for the analysis of some compounds without using additional gas supplies, high temperatures are still required in these methods.

It was also observed that protein charge distribution shifted with the change of the voltage for on-demand nanoESI. For example, myoglobin peaks at higher charge states became more abundant when the voltage increased from 350 to 430 V (Figure 3a). At 450 V, the charge distribution similar to that of regular nanoESI was obtained. For cytochrome C, a similar observation was obtained (Figure 3c) with a spectrum recorded at 600 V similar to that of regular nanoESI. Because of the denaturation of proteins during the nanoESI process with higher spray voltages, abundant peaks with higher charge states were usually observed in regular ESI or nanoESI. It should be noted that voltages higher than 600 V were required for the onset of regular nanoESI even when the tip was inserted into the inlet tubing. Using the on-demand nanoESI method, the generation of high-velocity gas flow may benefit to the ionization through production of smaller droplets.[26,29]

Analysis of Small Volume Samples. With the on-demand feature for low sample consumption during analysis, single-cell analysis was explored using the method developed in this work. Low-flow ESI-MS has been developed as a useful tool for the characterization of chemical information in various types of single cells.[30,31] The sample from a single cell is usually at very low volumes (pL−nL levels), which makes it

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Anal. Chem. 2021, 93, 6003−6007

challenging to perform multiple MS and MS/MS experiments. In on-demand nanoESI, the spray consumption of the sample in the glass tip can be synchronized with the injection of the ions into the mass spectrometer. Figure 4a shows the comparison of the sample consumption for MS analysis between on-demand and regular nanoESI. With a sample consumption corresponding to 1 s spray for regular nanoESI, at least 20 MS or MS/MS experiments can be performed using he on-demand nanoESI method, making it suitable for analysis of samples of extremely small volumes. It is worth noting that, different with pulse voltage[32] and piezoelectric dispensing methods,[33] the on-demand nanoESI method was convenient to control and did not require any additional electronics for control or synchronizing.

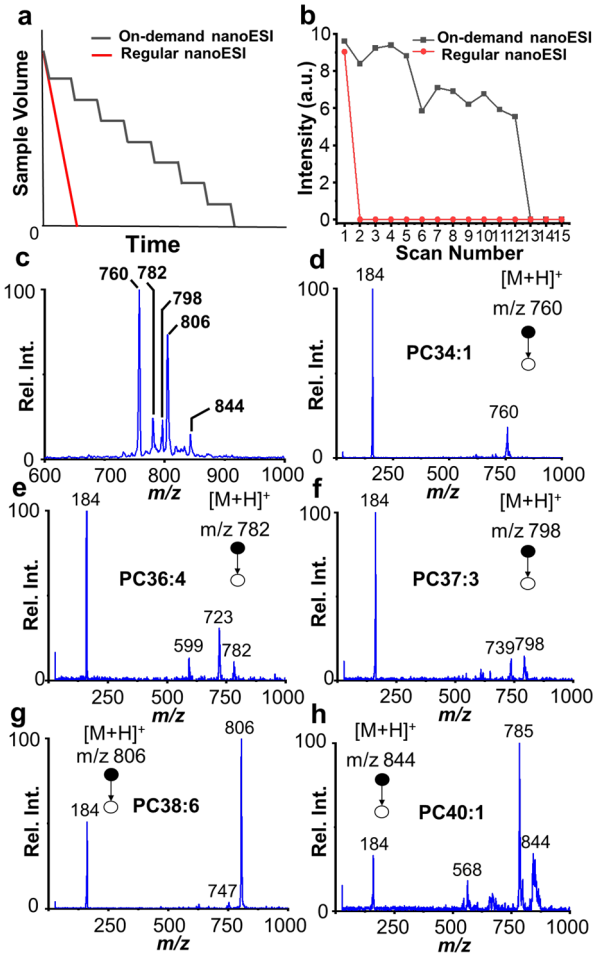

Figure 4. (a) Schematic of sample consumption by on-demand nanoESI and regular nanoESI along with time of ionization. (b) Comparison of the number of scans for fish egg lipid analyses by regular nanoESI and on-demand nanoESI. (c) Profile of phospholipids from a fish egg by on-demand nanoESI MS in positive ion mode. (d–h) MS/MS spectra of phospholipid species in the same fish egg.

The proof-of-concept demonstration was done by analyzing phospholipids in a Medaka egg cell (see details in the Supporting Information and Figure S10). At least 12 MS measurements could be performed for each sample at 600 V by the on-demand MS analysis method (Figure 4b and Figure S11). When using regular nanoESI, only one MS measurement could be performed for each sample (Figure S10c). The on-demand nanoESI MS was applied to profiling phospholipids as well as performing MS/MS for analysis of multiple phospholipid subclass species in the same single fish egg cell (Figure 4c–h). This would be useful for qualitative analysis for lipid metabolism studies. By using glass tips with smaller sizes,[6,31,34,35] it would be able to analyze samples with lower volumes. Because of the complication of cell manipulation, instrumentation, and analytical methodology, the single-cell MS methods are still limited in analytical laboratories. Miniature MS systems with simplified sample preparation procedures, such as the on-demand method developed in this work, will make it possible to transfer MS-based single-cell analysis methods into most biomedical laboratories for various biological or clinical applications.

CONCLUSIONS

In conclusion, a novel ionization method was developed based on discontinuous injection-induced ESI. Sensitive nanoESI MS analysis was realized by applying ionization voltages as low as 200 V. The on-demand nanoESI method was proven to be softer for the ionization of proteins, which would be a good alternative to native ESI methods. With an on-demand injection fashion, the method also allowed for performing multiple MS and MS/MS experiments in an extremely low-volume sample such as single cells by miniature MS system.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.analchem.1c00575.

Instrumentation and chemicals (Figure S1). Comparisons of on-demand nanoESI and regular nanoESI (Figures S2–S5). Investigation of effect of voltages (Figures S6–S7). Simulation of velocity and flow rate of induced air flow. Effects of glass tip sizes and insertion depths (Figures S8 and S9). Analysis of single fish eggs (Figures S10 and S11). (PDF)

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Notes

The authors declare the following competing financial interest(s): Z.O. is the founder of PURSPEC Technologies, Inc., which is developing the miniature mass spectrometry system.

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