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Novel On-Chip Nebulizer for Reliable ESI and icIEF-MS Based Charge Variant Identification of Biopharmaceuticals

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OVERVIEW

We present a versatile microfluidic chip with on-board nebulization to facilitate stable, long-lasting ESI spray, enabling reliable coupling of iCIEF to mass spectroscopy for systematic analysis of biopharmaceuticals and other biologic molecules. The nebulizer-assisted spray also facilitates efficient priming and exchange of reagents from the microfluidic chip using a wide range of flow rates.

INTRODUCTION

While microfluidic technology provides an elegant means to integrate liquid-phase separation methods with mass spectrometry (MS), there are special challenges when coupling to imaged capillary isoelectric focusing (iCIEF), which includes not only maintaining the focused samples but also the subsequent mobilization of the separation to the MS. On-board gas nebulizers were added to an integrated microfluidic chip to efficiently address these challenges. The increased aerosolization of expelled liquid facilitates stable electrospray ionization at 1-2 µL/min - low flow rates that facilitate sensitive detection of charge variants. Furthermore, nebulizer-assisted spray can accommodate high flow rates and stable uninterrupted spray, facilitating rapid sample loading, reagent priming and exchange even while the chip is positioned away from the MS orifice and without an applied electric field.

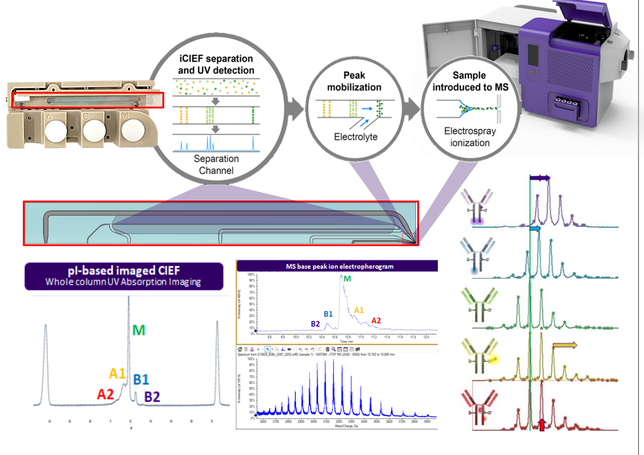


Figure 1. Integrated iCIEF-MS workflow. Our microfluidic chip (highlighted in *upper left*) is housed in a cartridge with reservoirs and ports that interface with the larger system shown on the *upper right.* Docked to the mass spectrometer, the combined IntaBio and ZenoTOF 7600 iCIEF-MS system automatically delivers controlled flows of reagents, applies voltages for the on-chip iCIEF separation, and positions the microfluidic chip near to the MS inlet for electrospray ionization. The workflow yields UV absorbance images of focused peaks (*lower left*), followed by an ESI-MS results with ion chronogram peaks (*middle*) that can be correlated with the CIEF image and underlying spectra (*lower middle*) that can be deconvoluted for identification of charge variants by MS. (*lower right*)

MATERIALS AND METHODS

Previously, we reported an integrated microfluidic chip with a separation channel for iCIEF along with tributary channels for anolyte, catholyte, and mobilizer reagents [1] (Fig. 1). All liquids, including mobilized separation, are expelled from the chip via an orifice at one of the chip's corners.

CIEF separation is performed in the central, longitudinal channel and is imaged via UV absorption. Following completion of this on-chip sample separation and focusing, analytes are chemically mobilized so they can be electrophoretically driven down the channel towards the orifice where the separated sample is hydrodynamically expelled from the chip at the corner orifice and delivered to a mass spectrometer via electrospray ionization. This spray is predominantly composed of a pressure driven 25% acetic acid acetonitrile solution. For MS detection of ions of interest, the tip of the chip is dynamically held at a constant 5.5 kV potential.

A pair of on-chip gas channels have been added on either side of the orifice to deliver high speed, shearing gas flow on either side of the exiting liquid. The nebulizers enable the efficient aerosolizing of expelled liquid and support both low-flow ESI as well as efficient, high-flow sample priming and reagent exchange. The orifice tip itself was shaped to an apex and rendered hydrophobic, focusing the electric field while substantially restricting the wettable area around the orifice (Fig. 2).

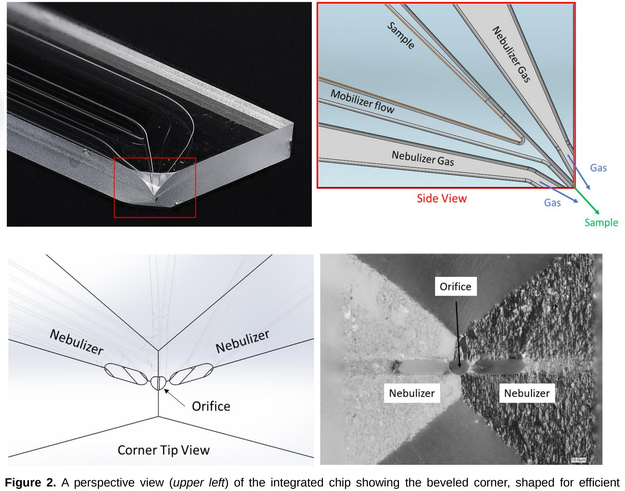


Figure 2. A perspective view (*upper left*) of the integrated chip showing the beveled corner, shaped for efficient electrospray. The sample orifice is located at the apex of this tip and is accompanied by two streams of nebulizer gas flow on either side (*upper right*). The *lower* panels show close-ups of the shaped tip immediately surrounding the orifice at the chip corner.

RESULTS

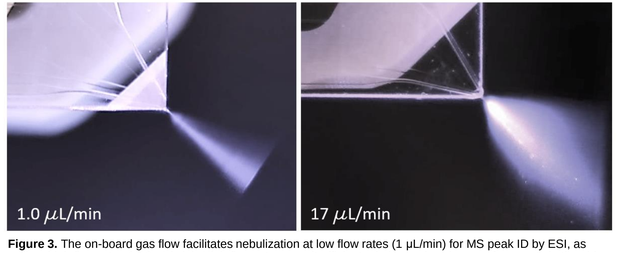


Figure 3. The on-board gas flow facilitates nebulization at low flow rates (1 µL/min) for MS peak ID by ESI, as well as high flow rates (>10 µL/min). High flow rates permit efficient priming and exchange of reagents in the chip while the overall chip and cartridge are retracted from the mass spectrometer inlet orifice between samples.

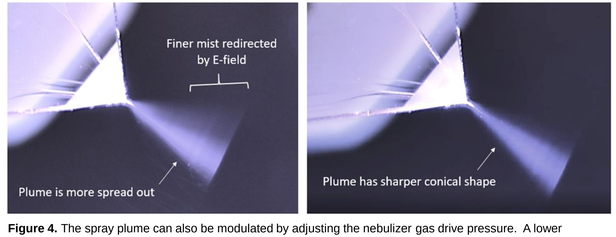


Figure 4. The spray plume can also be modulated by adjusting the nebulizer gas drive pressure. A lower nebulizer gas pressure (*left*) results in a wider plume, including a region of fine mist, while a higher pressure tends to focus spray plume into a narrow cone (*right*).

Nebulization Enables Robust and Steady Spray

While the nebulizers shown do not ringfence the orifice, as co-axial sprayers do, the use of high-speed gas at near sonic speeds is adequate to maintain spray plumes with stable and reproducible shapes and positions. With the hydrophobic, sharpened apex around the orifice, exiting liquid is confined to a small area with a correspondingly small Taylor Cone, preserving temporal and spatial resolution of separated sample being expelled from the chip. On-chip-nebulizer-assisted electrospray has been able to deliver a wide range of liquid flow rates for reagent compositions. Imaging of the illuminated electrospray envelope reveals a distinct region of fine droplets readily sampled by the mass spectrometer when spray is directed at an oblique angle relative to the MS inlet (Figs. 3, 4). Mass spectra of infused samples show stable and homogenous sprays across a wide range of flow rates and voltages, confirming the versatility and benefits of nebulized-assisted ESI. (Fig. 5, 6)

Efficient Sample Delivery and Reagent Exchange

To take advantage of the integrated iCIEF-MS chip, developing short, efficient workflows that can repeatedly used to perform rapid full assay is critical. Here nebulization provides another advantage: even when retracted from the MS source and in the absence of an applied electric field, nebulized spraying of liquid reagents can be used to efficiently move liquids through the chip during sample loading, priming and cleaning with flexibility of using a wide range of flow rates—from 1 to over 10 µL/min. (Fig. 3)

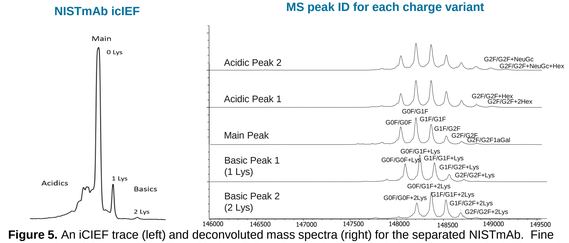
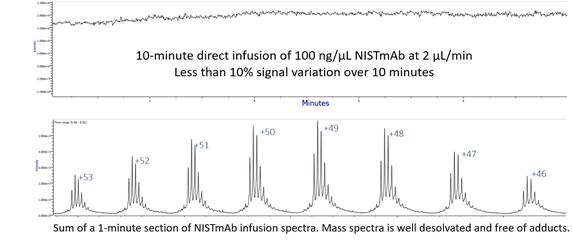


Figure 5. An iCIEF trace (left) and deconvoluted mass spectra (right) for the separated NISTmAb. Fine nebulization of the sample assists with the efficient desolvation of ions during electrospray ionization and analysis by MS, enabling detection of low abundance charge variants such as the antibody with two C-terminal lysines.



Sum of a 1-minute section of NISTmAb infusion spectra. Mass spectra is well desolvated and free of adducts.

Figure 6. Example of nebulizer-assisted ESI infusion of NISTmAb from the IntaBio chip with no separation

CONCLUSIONS

Nebulizer-assisted electrospray has been integrated with a microfluidic chip that couples iCIEF to mass spectrometry. Provided as a pair of high-speed gas jets on either side of the chip's exit orifice where liquid sample is expelled from the chip, nebulization helps to stabilize electrospray and maintain a steady sample flow rate without the reliance on an applied electric field.

The efficiency of nebulizer-assisted ionization confers numerous benefits:

1. Steady spray and efficient ionization of separated charge variants for identification of proteoforms
2. Sprayer can be optimally positioned with respect to the MS inlet to maximize sensitivity
3. Nebulization enables quick reagent exchange and priming of the microfluidic chip, allowing users to develop short, efficient workflows for their assays.

REFERENCES

[1] Mack *et. al.*, *Electrophoresis*, 40: 3084-3091

Data were deconvoluted with the IntaBio icIEF-MS workflow in BYOS from Protein Metrics.

PROTEIN METRICS