Sensitivity Improvement for Bottom-up Proteomics using Silicon Microfluidic Chip-Based Multinozzle Emitter Arrays at Capillary Flow Rates

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ABSTRACT

Purpose: The purpose of this work is to characterize the performance of microfabricated monolithic multinozzle (M3) emitter arrays, operated at capillary flow rates, relative to single nozzle emitters.

Methods: Two M3 emitter designs were fabricated and characterized. The number of nozzles in each design was varied though the total array height was kept constant to maintain high sampling into the slotted inlet of the Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribrid™ mass spectrometer.

Parallel reaction monitoring (PRM) experiments were carried out to assess the lower limits of quantitation (LOQ) as a function of the emitter type and flow rate. Data dependent acquisition (DDA) was employed to compare protein and peptide identifications across various experimental conditions.

Results: Operating at 1.5 µL/min, both M3 emitters delivered LOQs of amol and more than 4,200 protein groups identified from a HeLa cell digest using a 60 min gradient. Representing a 3- to 4-fold sensitivity increase relative to a single nozzle emitter operating at the same flow rate, the results were found to provide sensitivity identical to that which is observed at nano flow rates.

INTRODUCTION

It is well established that the sensitivity of liquid chromatography coupled to mass spectrometry (LC-MS) via electrospray ionization (ESI) increases significantly with decreasing column diameter and reduced flow rate; empirically, the relationship has been shown to follow a power law function [1]. One straightforward approach to maintain the sensitivity delivered at nano flow rates, while operating at substantially higher flow rates, is to distribute the eluent of the LC among discrete channels of a multinozzle emitter. In doing so, the spray current theoretically scales with the square-root of the number of nozzles [2]. This approach, previously shown to promote higher sampling of analyte ions into the first vacuum stage of the mass spectrometer, was evaluated at capillary flow rates herein.

MATERIALS AND METHODS

Sample Preparation

For PRM experiments, 6x5 peptide mixture (Promega) was spiked into 200 ng/µL HeLa cell protein digest (Thermo Fisher Scientific) at concentrations of 1 amol/µL - 10 fmol/µL. For DDA experiments, 1 µg/µL HeLa cell protein digest was prepared. In all cases, samples were prepared in water containing 0.1 % formic acid and 2% acetonitrile.

Test Method(s)

M3 emitter performance was characterized by reversed-phase LC separation using a Thermo Scientific™ UltiMate™ 3000 RSLCnano UHPLC system and mass spectrometry detection on a Fusion Lumos Tribrid mass spectrometer equipped with Thermo Scientific™ Nanospray Flex™ NG ion source and Thermo Scientific™ EASY-Spray™ NG ion source. For PRM and DDA experiments, 1 µL of sample was loaded on-column via full loop injection. Capillary flow separations were carried out at 1.5 µL/min using a 15 cm x 150 µm Thermo Scientific™ Acclaim™ PepMap™ column. A discrete column was employed to directly assess the performance of M3 emitters relative to a single nozzle emitter (ES791, 7 µm internal emitter diameter). In addition, the same samples were analyzed using two Thermo Scientific™ EASY-Spray™ PepMap™ columns of varying length (50 cm x 75 um (ES803, 7 µm internal emitter diameter) and 15 cm x 75 µm (ES804, 7 µm internal emitter diameter)) operated at 300 nL/min. In all cases, the gradient ramped from 3% to 40%B (where [A]: 0.1% formic acid in water, [B]: 0.1% formic acid in 80:20 acetonitrile:water).

For PRM experiments, a 30 min gradient was used. The Orbitrap Fusion Lumos instrument acquired tMS2 scans using HCD with Orbitrap detection at 50k, an AGC target of 1e6, and an injection time of 110 ms. The quadrupole was used to isolate precursors at m/z 0.7.

For DDA, a 60 min gradient was employed. The Orbitrap Fusion Lumos instrument acquired a full scan at 120k and an AGC target of 2e6. MS2 scans were acquired via HCD with detection in the ion trap using rapid (20 ms injection time) and turbo (10 ms injection time) scan rates using a quadrupole isolation of m/z 1.2, an AGC target of 2e4, and a dynamic exclusion of 7 s.

Data Analysis

DDA files were searched in Thermo Scientific™ Proteome Discoverer™ 2.2 software using SEQUEST® HT and Percolator to filter results to a 1% FDR. PRM files were analyzed using Skyline 3.7.0.11317.software.

Table 1. Emitter design parameters and experimental operating conditions.

	M3-8	M3-5	single nozzle
Number of active nozzles	8	5	1
Number of total nozzles	10	7	1
Internal nozzle cross-section, µm²	5 x 5 = 25 µm²	10 x 10 = 100 µm²	π x (7/2)² = 38.5 µm²
Effective flow rate per nozzle (at 1.5 µL/min), nL/min	188	300	1500
Sheath gas flow rate, L/min	0	1.0	-
Spray voltage, kV	4.5	4.5	2.0
Emitter-inlet distance, mm	3.0	3.0	1.0

RESULTS

M3 emitters were fabricated using photolithography and deep reactive ion etching from two fused silicon wafers that form a quadrangular internal channel. These devices have been previously shown to create sufficient electric field strength to generate and sustain uniform Taylor cones for designs containing up to 80 nozzles [3]. However, inter-nozzle spacing on the order of a few tens of microns resulted in intra-nozzle interactions that principally limited the collective sensitivity improvement. In the present work, we limit the scope to designs featuring ≤10 nozzles.

Figure 1. Photograph of the M3 emitter product. (a) M3-8 shown with respect to the slotted ion inlet of the mass spectrometer (b), M3-8 (c), and M3-5 (d). Note that one nozzle on each side is dry and only serves to shape the electric field.

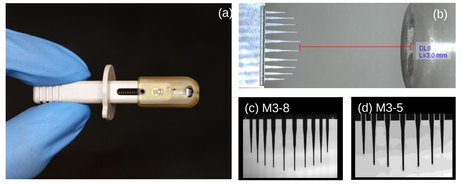
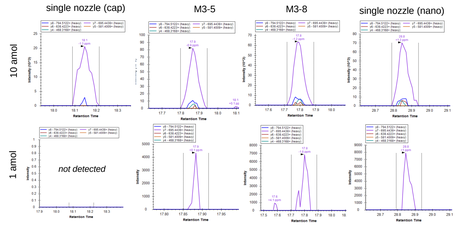


Figure 2. Chromatograms for VVGGLVALR as a function of concentration and emitter type, as indicated below.



The total internal volume of the M3 emitter is ~7 nL and therefore does not significantly contribute to peak broadening at the flow rates employed. The results shown in Figure 2 demonstrate comparable chromatographic peak shapes for all emitter types.

Figure 3. Calibration curves of the single nozzle emitter compared to M3 emitters as measured by the 6x5 peptide dynamic range mixture. The flow rate of each experiment is indicated in the legend.

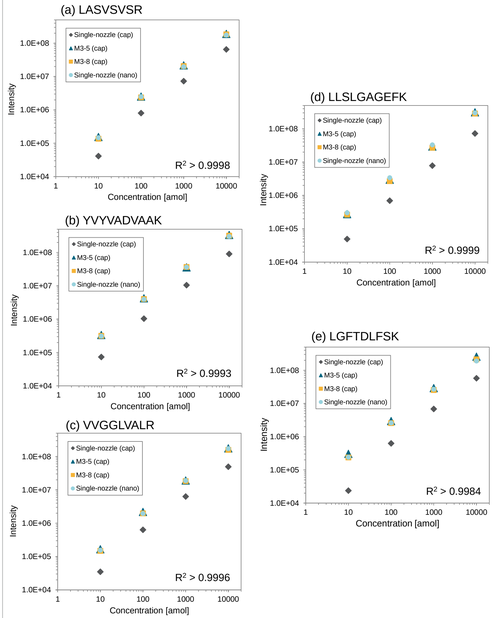


Figure 4. Peak area ratio as determined by the slope of the calibration curves, for M3-5 and M3-8 relative to the single nozzle operating at 1.5 µL/min.

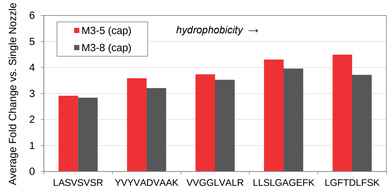
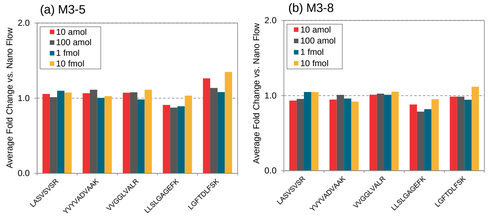


Figure 5. Average fold change, as determined by the slope of the calibration curves, for M3-5 (a) and M3-8 (b) relative to the ES803 single nozzle emitter operating at nano flow rates.



Figures 3-5 demonstrate that the expected 4-fold decrease in sensitivity at capillary flow rate (based on the increase in column diameter) is compensated by an increase in ionization efficiency and/or atmosphere-to-vacuum transfer using M3 emitters. Note that the most hydrophilic peptide in the 6x5 mixture (VTSGSTSTSR) did not bind to the column and was therefore excluded from analysis. Figure 5 clearly shows that the sensitivity of both M3 emitters operating at capillary flow rate is equivalent to a single nozzle emitter operating at nano flow. The results are in good agreement with past experimental results predicting a 3-fold decrease in sensitivity upon increasing the flow rate from 300 nL/min to 1.5 µL/min [1].

Table 2. Lower limit of quantitation for the dynamic range peptide mixture. Values in parenthesis correspond to the coefficient of variation at the LOQ.

LOQ at CV <15%, amol (CV value at LOQ, %)					
	LASVSVSR	YVYVADVAAK	VVGGLVALR	LLSLGAGEFK	LGFTDLFSK
single nozzle (cap)	100 (5.2)	10 (9.8)	10 (14)	10 (9.7)	100 (5.2)
M3-5	10 (4.7)	10 (13)	10 (2.3)	10 (7.6)	10 (2.6)
M3-8	10 (3.3)	10 (4.0)	10 (4.3)	10 (7.5)	10 (9.2)
single nozzle (nano)	10 (5.7)	10 (6.9)	10 (4.0)	10 (4.1)	10 (4.4)

Figure 6. Protein group identifications in 60 min with varied ion trap scan rates and emitter types, as indicated below. Unless explicitly noted, the column length was 15 cm.

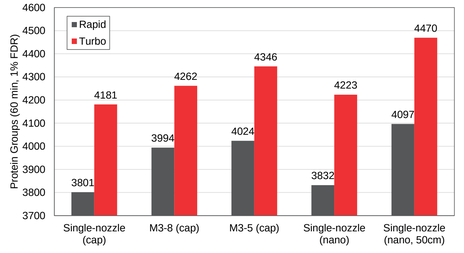


Figure 7. (a) Abundance ratios of peptide groups for the M3-5 emitter relative to the single nozzle emitter. Distributions are shown at 10 min intervals along the gradient. Results were filtered for CV values <15%. (b) Frequency distribution of MS2 peptide spectral match (PSM) intensities. (c) Average isolation interference shown as a function of intensity.

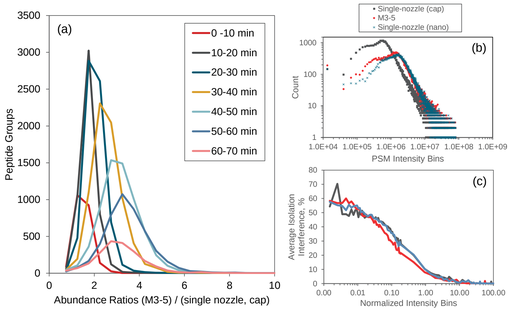


Figure 7a and 7b demonstrate that both MS1 and MS2 ion abundances increased with the M3-5 emitter, respectively. As the gradient was ramped, the mean abundance ratio increase was accompanied by an increase in the width of the distribution. Figure 7c shows that the peak purity was not significantly altered by the emitter type.

CONCLUSIONS

- Both M3 emitter designs evaluated offered similar performance. One explanation for this behavior is that upon adding additional nozzles, the decrease in effective flow rate per nozzle is offset by an increase in intra-nozzle interactions.
- Operating at 1.5 µL/min, M3 emitters delivered: (1) lower limits of quantitation of 10 amol, (2) more than 4,200 protein groups identified from a HeLa cell digest using a 60 min gradient, (3) 3- to 4-fold sensitivity increase relative to a single nozzle emitter operating at the same flow rate, (4) identical sensitivity to nano flow.
- The average gain in sensitivity for M3 emitters was found to increase along the gradient. Future experiments to further elucidate the ESI mechanisms at work may be useful to guide further development.

REFERENCES

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