A Hybrid Microchip/Capillary Electrophoresis Mass Spectrometry Platform for Rapid and Ultrasensitive Bioanalysis

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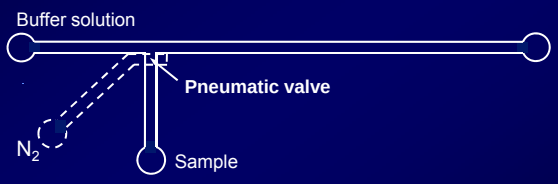
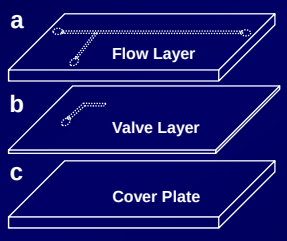
Hydrodynamic sample injector with a pneumatic valve on a polydimethylsiloxane (PDMS) microchip for capillary electrophoresis (CE) separations

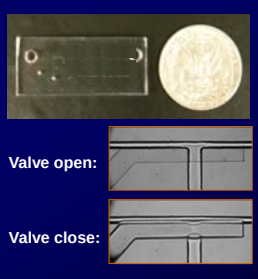
Microchip Design:



Fabrication:



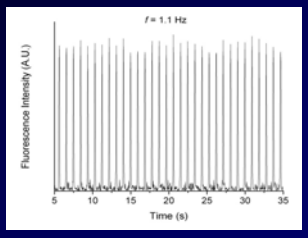
Device:



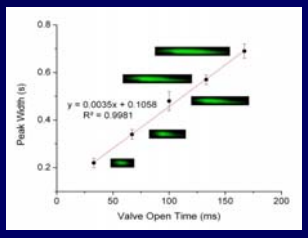
Sample Injections:

Characteristics of the microchip injector*

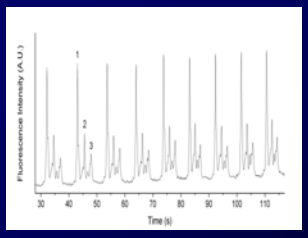
a) Reproducible, lossless and unbias injection



b) Flexible injection volume (100 pL – 10 nL)



c) Multiplexed CE separation

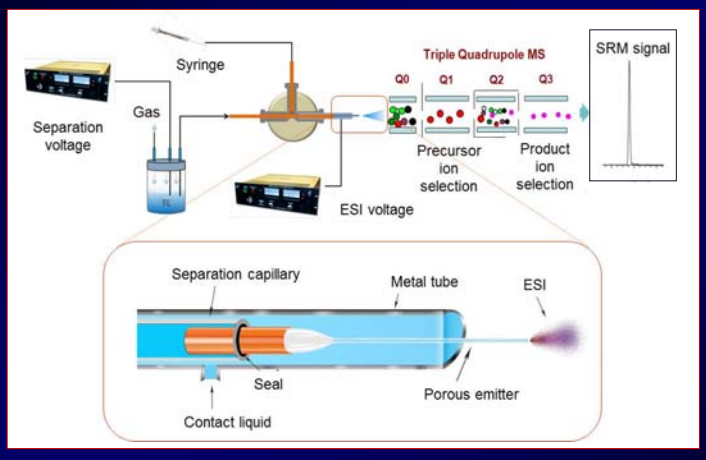


Sample: 333 nM of fluorescein 5(6)-isothiocyanate (FITC) labeled Gly (1), Phe (2), and Arg (3). Experimental conditions: E = 1000 V/cm; Injection frequency: ~ 0.1 Hz; Injection time: ~ 67 ms

Major problem: Poor separation quality due to PDMS surface interactions with analytes

* X. Sun et al., *Electrophoresis, 32, 1610-1618* (2011).

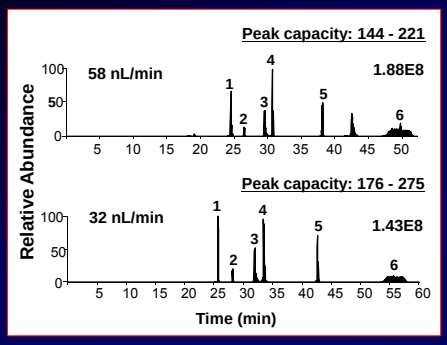
A sheathless interface for online CE-MS



- **Larger i.d. separation capillary(100 µm i.d./ 360 µm i.d., 95 cm long) for large sample loading and small i.d. ESI emitter capillary (20 µm i.d./ 90 µm o.d.) for stable nanoESI operation**
- **Electric contact for ESI voltage through conductive liquid enclosed in a short metal tube and etched porous wall of the emitter capillary**

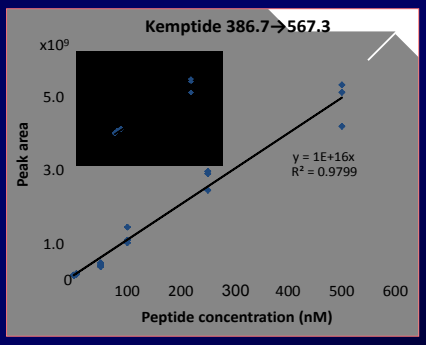
Performance of the sheathless CE-MS*

a) High separation quality:



CITP/CZE-SRM MS at two different ESI flow rates, The labeled peaks from 1 to 6 are kemptide, BSA peptide III, BSA peptide II, angiotensin II, BSA peptide I, and leu-enkephalin, respectively

b) High sensitivity:



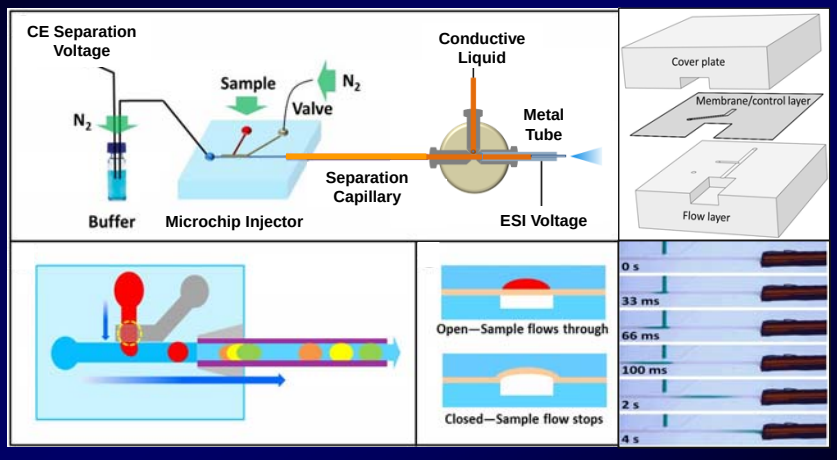
Limit of quantitation for CITP/CZE-SRM MS analysis of kemtide: 10 pM with total sample loading of 25 attomoles (2.5 µl), ESI flow rate: 58 nL/min

Sample: 50 nM each petide spiked into 50 nM BSA digest matrix

Major problem: Low throughput due to the interruption of CE separation for offline sample loading

* C. Wang et al., *Anal. Chem., 85, 7308–7315* (2013).

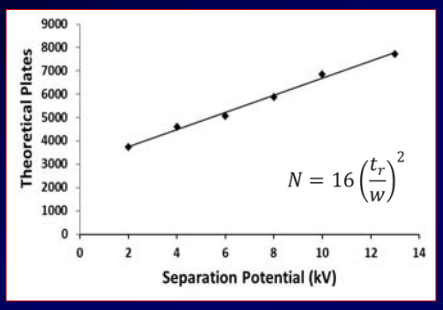
A Hybrid Microchip/CE system that combines hydrodynamic sample injection, high resolution CE separation and high sensitivity CE-MS*



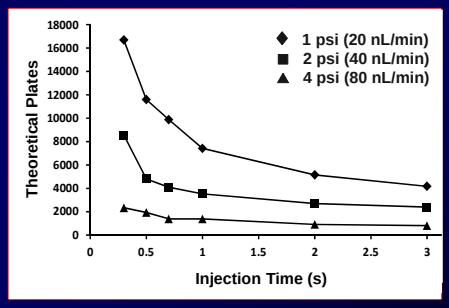
- Separation capillary: 30 µm i.d., 140 µm o.d., 25 cm long; PDMS microchip: 25 µm tall X 100 µm wide rectangular control layer channel; ~10 µm tall X100 µm wide rounded bottom flow layer channels; CE buffer: 9:1 of 0.1 M acetic acid in water: methanol

* R. T Kelly et al., *Anal. Chem.,* DOI: 10.1021/ac501910p (2014).

The separation performance evaluation of the Hybrid Microchip/CE system coupled with a triple quadrupole MS via the sheathless nanoESI interface

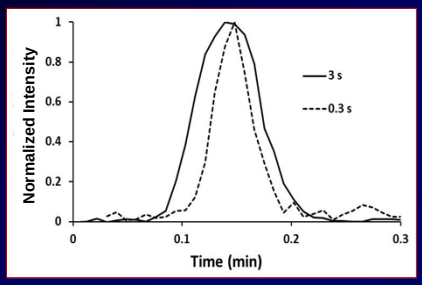


Theoretical plates at different potentials for leucine enkephalin. Injection and elution pressures: 2 psi (40 nL/min); Injection time: 0.5 s (~330 pL). Sample concentration: 10 μM in CE buffer

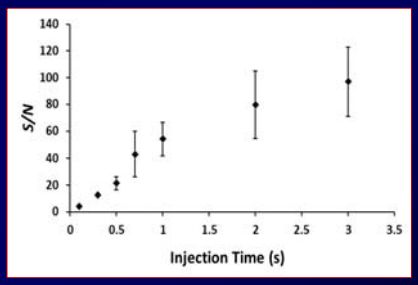


Theoretical plates at different injection times for leucine enkephalin. Separation potential : 13 kV; Eluting and injection pressures : 1 psi (♦), 2 psi (■) and 4 psi (▲)

Tradeoff between separation efficiency and *S/N* for the hybrid microchip/sheathless CE MS instrument

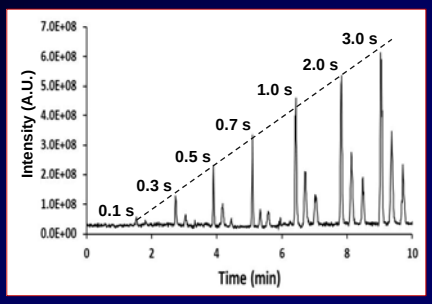


MS peak width for Kemptide at two different sample injection times. Injection and eluting pressures: 2 psi (40 nL/min).
Sample concentration: 10 μM in CE buffer

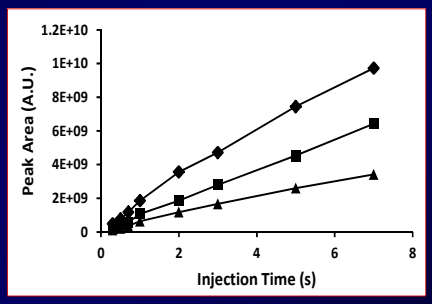


S/N vs. injection time for kemptide. The eluting and injection pressures were both 2 psi. Error bars are standard deviation from 3 replicate CE-SRM MS analyses

Multiplexed CE separation from unbiased and lossless sample injection allowing quantitative MS detection



MS peak intensity from multiplexed CE-MS analyses of repeated injections at different injection times. Sample: a mixture of kemptide, angiotensin II and leucine enkephalin, 10 μM each in run buffer (in order of highest to lowest mobility); Sample injection interval: 1.25 min. Injection and elution pressures: 2 psi (40 nL/min)



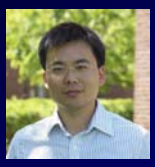
Peak areas at different sample injection time for kemptide (♦), angiotensin II (■) and leucine enkephalin (▲). Injection and elution pressures: 2 psi (40 nL/min)

Conclusions

- Hybrid microchip/sheathless CE-MS allows unbiased and lossless sample injection, high resolution CE separation, and quantitative high sensitivity MS detection
- Computer-controlled pneumatic microvalve enables flexible and reproducible sample injection volumes (picoliters to nanoliters) based on valve open time and injection pressure
- Uninterrupted CE operation during sample injection allows multiplexed CE separations for higher throughput CE-MS analysis
- Potential to combine with nanoflow capillary LC for fully automated 2-dimensional LC-CE separations followed by high sensitivity MS detection

Acknowledgements

CE-SRM MS technology development: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