CZE-nanoESI-SLIM-IMS-MS Platforms for Comprehensive, Ultrasensitive Proteome Analyses


Overview
- CZE separations were performed on an in-house constructed CZE system.
- The CZE interface [1] employed a 15 µm i.d. tapered borosilicate glass emitter and an electrokinetically pumped sheath liquid with 300 µm between the etched capillary exit and the ESI emitter.
- Experimental conditions used were as follows:
  - CZE-ESI of 1 µm phosphopeptide isomers (Figure 2): 30-cm long, 30 µm i.d. capillary with 200 mM formic acid as background electrolyte, 10 mM formic acid, 20% methanol as sheath liquid (SL); 19 kV separation.
  - CZE-ESI for 10 µM BSA digest (Figure 3): 28-cm long, 20 µm i.d. capillary with 100 mM acetic acid as BGE and 20 mM acetic acid, 20% methanol as SL; 19 kV separation.
  - CZE-ESI of intact proteins in acidic condition (Figure 4A-C): 45 cm long 50 µm i.d. capillaries, coated with 2 mg/ml Polybrene; with 100 mM formic acid as BGE. 10 mM formic acid, 20% methanol as SL; 19 kV separation.
  - Native CZE-ESI separations for intact proteins (Figure 4D): 35 cm long 20 µm i.d. capillary with 100 mM ammonium acetate at pH 7.1 as BGE. 100 mM ammonium acetate at pH 7.1 as SL; 15 kV separation.

Introduction
Progress towards comprehensive proteome characterization relies on the development of high resolution front and separation techniques, efficient ionization and improved ion transmission mass spectrometric analysis. In this work we couple CZE-nanoESI with various IMS platforms, including:
- a 1 mm drift tube-IMS-MS
- a high charge capacity prototype SLIM-TW-IMS-MS platform.

Methods
CZE separations were optimized for three separation dimensions which can be optimized for different separation conditions including:
- Ultra-sensitive and efficient separations
- Enhancement of peak capacity

Separation & structural characterization of intact proteins in denatured and native conditions

Results
Enhancing peak capacity for peptide separations using extended path length SLIM-TW-IMS-TOF-MS
- Phosphopeptide isomers partially separate in both CZE and IMS dimensions (Figure 2).
- A high charge capacity prototype SLIM-TW-IMS-MS is compatible with fast CZE-nanoESI separations.

Intact protein separations and structural characterization using CZE with DT-IMS-qTOF-MS
- CZE allows protein separation in native states (Figure 4) for correlation with IMS structures.

CE peak parking for longer analysis times
- NanoESI sheath flow CE-IMA interface enables parking of the CE separation while maintaining a stable electrospray.

Conclusions
- CZE, IMS and MS provide three separation dimensions which can be optimized for specific analytical problems
- CZE-IMS-MS enhances the peak capacity of both peptide and intact protein separations, provides a tool for solution versus gas phase conformations studies, and allows investigation into background electrolytes and pH conditions
- The electrokinetic sheath flow of the CZE-nanoESI interface supports stable electrospray even without the addition of organic solvent, allowing analysis of native solution proteins and complexes
- The increased charge capacity of SLIM devices helps alleviate low injection volume limitations of fast, high electric field CE separations
- High resolution SLIM-MS separations enhance CE peak capacity and overcome quantitation limitations due to co-elution of the isomeric species
- CZE-nanoESI can be temporarily stopped without incurring sample losses or significant band broadening

Acknowledgements
Portions of this research were supported by grants from the National Institute of General Medical Sciences (P41 GM103493), National Institute of Environmental Health Sciences of the NIH (R01ES022190) and the Laboratory Directed Research and Development Program at Pacific Northwest National Laboratory (PNNL). PNNL is operated for the U.S. Department of Energy by Battelle

References
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CZE-ESI-capillary electrophoresis; MS-MSI-ion mobility; IMS-ion mobility spectrometry; SLIM-serial linear ion mobility; CZE-ESI coupled with the nanoESI sheath flow interface

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