Fast Chip-CEMS for Characterization of Protein/PTM Changes

Deborah R. Leon, Kshitij Khatri, Bo Yan, John R. Hasserick, Christian Heckendorf, Joseph Zaia, Catherine E. Costello, Mark E. McComb

Boston University School of Medicine, Boston MA

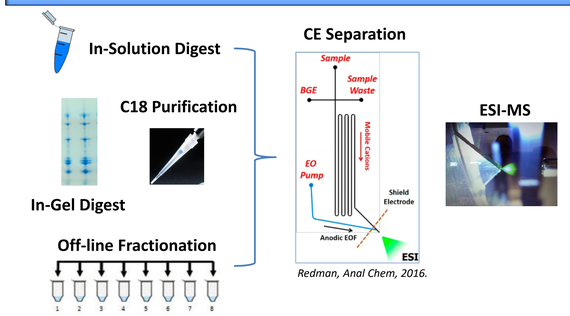
Overview

- Chip-CEMS offers the capability to analyze extremely small sample volumes, separate peptide and/protein isoforms and achieve very high analytical resolution in a fraction of the time of comparable HPLC-MS experiments.

CEMS

- Capillary electrophoresis mass spectrometry (CEMS) offers the most powerful means of acquiring on-line ESI-MS data from both peptide mixtures and intact proteins.
- Flow rates are directly compatible with ESI-MS and CE offers extremely high resolution in terms of theoretical plates, with peptide peak widths typically less than 1 second (fwhm).
- Recently, 908 Devices (Boston, MA) introduced a prototype commercial chip-based CEMS interface.
- We conducted a series of pilot projects to evaluate the capabilities of the interface and explored a wide range of different biomolecules to determine suitability for high-throughput bottom-up and top-down MS/MS for characterization of protein post-translational modifications (PTMs) and elucidation of proteoforms of intact proteins.

Applications of CEMS



Redman, Anal Chem, 2016.

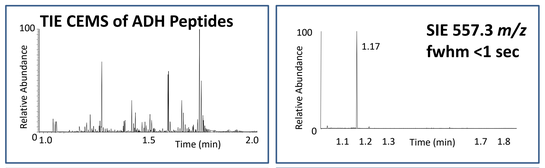
Approach

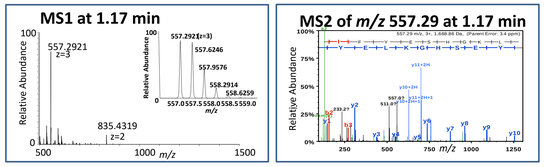
- CEMS employed a 908 Devices ZipChip™ CE-ESI interface.
- LCMS was performed using a Waters nanoAcquity equipped with an Advion Nanomate ESI source.
- MS and MS/MS analyses were carried out using Q Exactive, Q Exactive Plus or QExactive HF mass spectrometers (Thermo-Fisher).
- Peptides/proteins were made up in 50% organic solvent (ACN, MeOH or isopropanol) prior to CEMS; picomole to low femtomole sample loads were analyzed.
- Bottom-up samples included peptide standards, in-solution protein digests and in-gel protein digests; Top-Down analysis used protein standards and pre-fractionated yeast proteome samples.
- MS feature identification was enabled by analyzing the MS/MS data using Proteome Discoverer (Thermo-Fisher) and Mascot (Matrix Science) software.
- PTM analysis was performed using Scaffold (Proteome Software) and PEAKS Studio (Bioinformatics Solutions).
- Top down MS and MS/MS analysis was performed using BUPID (Boston University Protein Identifier) software developed in our laboratory at BUSM.

Initial CEMS Studies: ADH Peptides

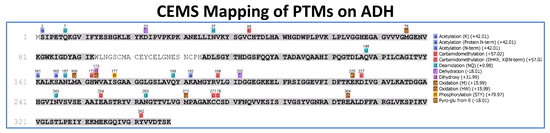
- A series of CEMS experiments were performed using peptide standards and a standard digest of alcohol dehydrogenase (ADH).
- We tried different chip designs, CE electrolytes and buffers in order to maximize CE separation efficiency.
- We explored different concentrations of sample and different injection times to maximize sample introduction while maintaining narrow peak widths in CE.
- We adjusted MS1 and MS2 parameters on the Q Exactive series mass spectrometers to account for narrow peak widths of CE in order to maximize the quality of MS1 and MS2 data and ensure adequate duty cycle and sufficient ion signal for quality MS2 data.
- MS parameters explored included: *m/z* range, max fill time, MS1 and MS2 resolution, AGC, MS2 trigger, lock masses. etc.

CEMS of a Digest of ADH





CEMS Mapping of PTMs on ADH



- CEMS of peptides from a digest of ADH yielded 2-min run times with >400 MS2 spectra and >200 confident peptide assignments. Peak widths were <1sec fwhm. Multiple PTMs were assigned.
- Setting the *m/z* range 500-1500 resulted in an increase in S/N compared with *m/z* 370-1800, but yielded a loss of small *m/z* precursors and a reduction in the number of peptides identified.
- Increasing the MS2 threshold to 5e5 resulted in less MS2 but yielded more confident assignments of higher quality MS2 data.
- Reducing the duty cycle to Top 5 allowed for more cycle time, improved MS1 TIE (SIE) and accommodated the narrow CE peaks.
- We achieved optimum performance with 50msec fill/transient at 17,500 resolution and top-5 MS2 with an MS2 trigger of 3e5 over an MS1 *m/z* range of 500–1500.

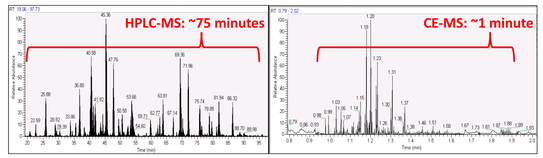
Evaluation of MS1 and MS2 parameters

Method	A11	B11	C11	D11	E11
Conc. µM	0.1	0.1	0.1	1	1
Trigger	1.00E+05	1.00E+04	3.00E+05	3.00E+05	3.00E+05
Top n	20	20	5	5	5
m/z range	370	370	370	500	370
Sequest Score	34	25	60	71	95
% Coverage	38	38	49	52	57
# PSM	17	15	24	20	29
Mascot Score	723	539	1046	972	1242
% Coverage	40	32	51	52	59
# PSM	18	12	25	20	30

CEMS vs. LCMS In-Gel Digest proteins

- We further explored the potential of CEMS by directly comparing analyses of identical samples processed using standard LCMS protocols in our laboratory.
- CEMS and LCMS were performed on peptides obtained from digests of protein mixtures and from in-gel digests of proteins.
- In-gel digests of proteins yielded results similar to in-solution digests of proteins.
- CEMS results were comparable to results obtained using LCMS which required significantly longer run times.

Example TIC and TIE from In-gel Digest Peptides



Peptides Assignments of In-Gel Digested Proteins

	CEMS			LCMS		
Protein	# Peptides	# MS2	% Coverage	# Peptides	# MS2	% Coverage
Ferritin LC	9	89	50	8	57	50
Glucose oxidase	18	49	40	17	69	36
Catalase	34	155	60	39	235	54
GAPDH	14	70	44	22	100	53
Trypsin Inhibitor	12	143	49	11	115	45
B-Lactoglobulin	13	191	60	18	144	75
Myoglobin	15	140	82	19	124	90
Cytochrome C	19	57	71	16	78	62

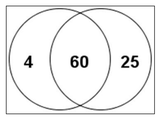
CEMS of Peptides from Protein Mixtures

- Protein mixtures were digested to peptides and analyzed by CEMS using short high speed (HS) and longer high resolution (HR) chips.
- HR chip CEMS run times were approximately 5 minutes, compared with 2 minute run times on HS chips, which resulted in a gain of approximately 7% more sequence coverage compared to HS chips.
- HR chip CEMS of the protein mixture yielded results comparable to those obtained using standard LCMS protocols.
- Again, sufficient high quality MS2 data were obtained from CEMS which allowed the identification of a number of PTMs.

CEMS of Peptides from a Protein Mixture: HS vs. HR Chips

Protein name	% Coverage HS Chip	HR Chip
Ferritin light chain	24	30
Glucose oxidase	12	18
Catalase	40	45
GAPDH	20	33
Trypsin inhibitor A	26	30
Beta-lactoglobulin	46	46
Myoglobin	50	60
Cytochrome c	47	55

Myoglobin % Coverage

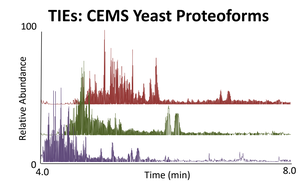


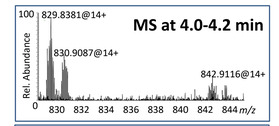
ITRB_SOYBN

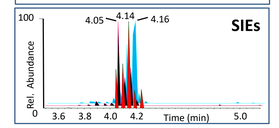
ITRA_SOYBN

CEMS for TopDown Proteomics

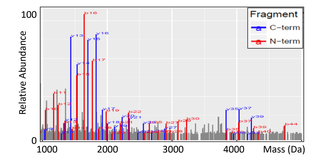
- We also explored the possibility for intact protein separation for nano-scale top-down MS2. Different electrolytes and chip designs were evaluated.
- We used optimized protein CEMS parameters to analyze yeast sub-proteomes. In brief: yeast samples were fractioned via 30-kDa ultrafiltration, cleaned up via C18 and analyzed by protein CEMS using protein top-down optimized MS and MS2 parameters.
- A single 4-min CEMS experiment yielded >250 protein/proteoform identifications including tentative assignments of PTMs.

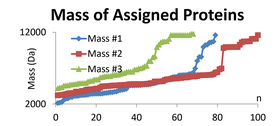






BUPID TopDown Assignment: HSP12



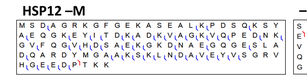


CEMS: BUPID Topdown Assignments of HSP12

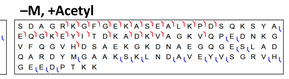
tag.score	tag.cov	scan	time	preMZ	preZ	preMass	fragcov	cov.n	cov.c
211.76	40.37	382	228.3	1658.6700	7	11603.69	0.44	0.00	0.44
254.35	46.79	384	230.5	1451.4600	8	11603.68	0.46	0.01	0.46
336.27	55.05	385	231.9	893.5200	13	11602.76	0.28	0.03	0.27

Proteoform Assignment by BUPID Topdown

HSP12 –M

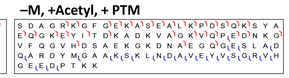


–M, +Acetyl



–M, +Acetyl, + Methyl

–M, +Acetyl, + PTM



Summary

- In conclusion we demonstrate the application of a new chip-based microfluidic CE interface for high resolution mass spectrometry for peptide and protein sequencing, for protein identification and characterization and for protein PTM characterization.
- See also poster WP 225 Microfluidic Capillary Electrophoresis – Mass Spectrometry for Glycomics and Glycoproteomics; Kshitij Khatri; Joshua A. Klein; John R. Haserick; Deborah R. Leon; Catherine E. Costello; Mark E. McComb; Joseph Zaia; Boston University, Boston, MA

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