Capillary Electrophoresis and Coupling with MS

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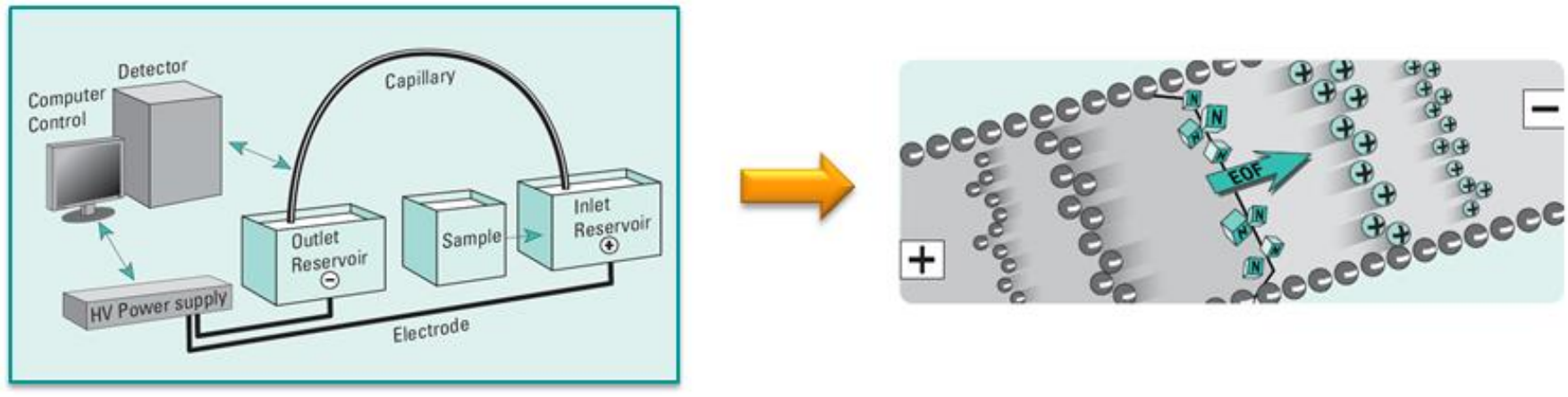
About the Author

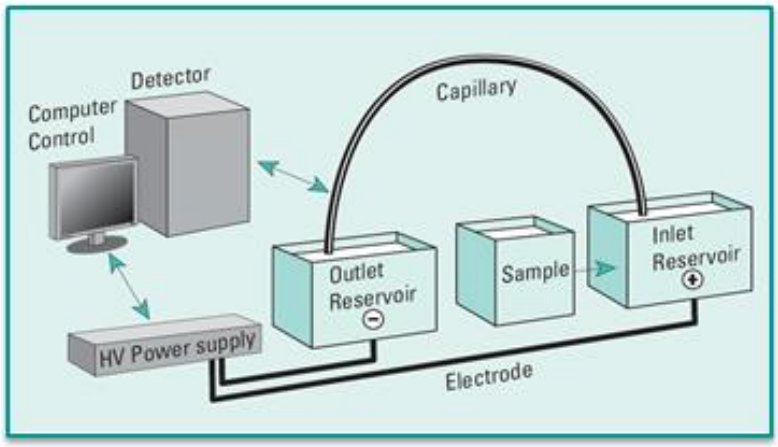
- Undergraduate and graduate studies at University of Amsterdam, 1964-1976. Majors in Organic Chemistry and Chemical Engineering
- Post-doctoral research at State University of Ghent, Belgium, 1977 and post-doctoral training Analytical Chemistry, University of Amsterdam, 1978-1979
- R&D Chemist, group & project Leader, R&D section manager, HPLC column and HPLC system development at Hewlett-Packard, Waldbronn, Germany, 1979-'99
- Since 2000, Agilent Technologies University Relations and External Scientific Collaborations Manager. Agilent Research Fellow since 2006
- Retired September 1, 2012. Since then, working on freelance basis. Visit my website at http://www.rozing.com e-mail: gerard@rozing.com

Capillary Electrophoresis Principle



- Solute separation is based on difference in their electrophoretic mobility (mass/charge ratio)
- Solutes move through the capillary by the combined action of electrophoretic mobility and electro-endo-osmotic flow (EOF, up to 3 mm/s)
- EOF has a flat velocity profile across capillary (in contrast to hydrodynamic flow through a capillary)
- Low zone broadening ➔ high separation efficiency
- Fused silica capillary 10-150 µm i.d.
- Sample volume variable; typically 5-100 nL
- Solvent is an aqueous, conducting electrolyte (BGE) solution; additives to modify selectivity of separation

Capillary Electrophoresis Modes of Operation



Modes of Capillary Electrophoresis	
Capillary Zone Electrophoresis (CZE)	Anion/Cations, low MW – high MW
Capillary Gel Electrophoresis (CGE)	Proteins, Oligonucleotides, RNA, DNA
Capillary IsoElectric Focusing (CIEF)	Peptides, Proteins
Capillary Isotachophoresis (CITP)	Peptides, Proteins, pre-concentration
Micellar Electrokinetic Chromatography (MEKC); Capillary Electrochromatography (CEC)	Chromatography; neutral molecules, chirals

Capillary Electrophoresis vs. HPLC

Pro's

- Very high separation power
- Only very small sample size required
- Versatile through multiple separation modes and variations
- Method of choice for ionized molecules
- Simple sample prep
- In general high recovery
- Separation principle orthogonal to reversed phase HPLC
- Highly automated instrumentation

Con's

- Method cannot be scaled like HPLC; limited to low i.d. capillaries
- Limited to very small samples
- UV-VIS detection limited sensitivity; pre-concentration methods req.
- Regarded to be a difficult method
- EOF, solute mobility depend on run buffer composition; difficult to predict
- Method optimization requires experience and knowhow; use of modelling is convenient

Capillary Electrophoresis ←→ (U)HPLC

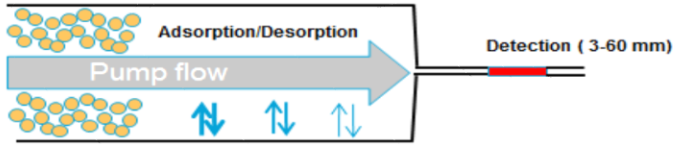
Chromatography► **complements** **◄Electrophoresis**

Separation based on differential partitioning between a mobile and a stationary phase

Chromatography:

Flow rate: constant (µL to ml/min)

Columns: packed with adsorption material
Separation: different adsorption/desorption behaviour
Peak width: depending on retention time

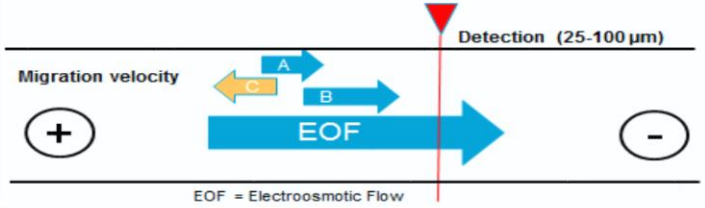


Separation is based on the differences in compound specific velocity in an electric field

Electrophoresis:

Flow rate: a) pH dependend capillary flow rate (nL/min)
b) compound specific migration

Columns: None , open tube (fused silica capillaries)
Separation : Compound dependent migration speed / directions
Peak width: depending on mobility of compound and buffer



Sample Capacity
Detector Options
Sensitivity
Reproducibility
Green Methods
Neutral Compounds
Mainstream Technique
Cost of Ownership
Biocompatibility

Large Molecules
Biocompatibility
Small sample Volume
Green Methods
Cost of Ownership
Sample Capacity
Reduced Sample Prep
High Resolution
Charged Compounds

Ideal — Ideal

Slide courtesy of Agilent Technologies

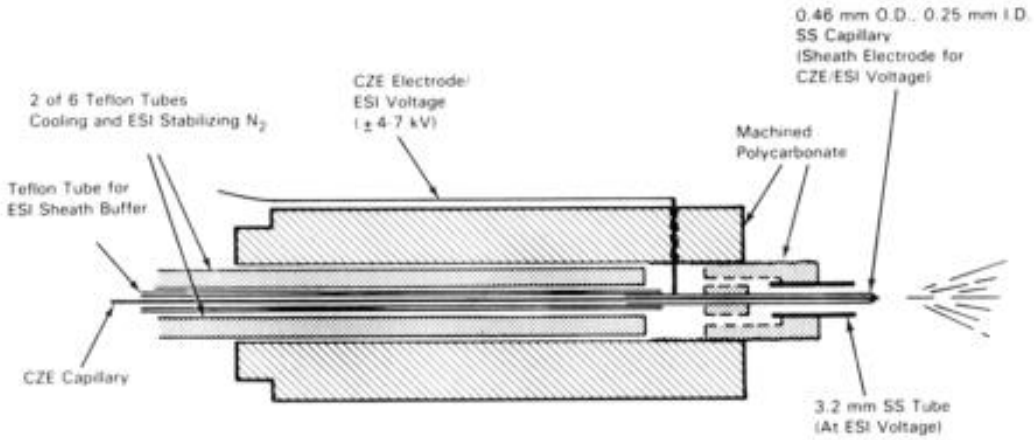
CE-ESI/MS Coupling

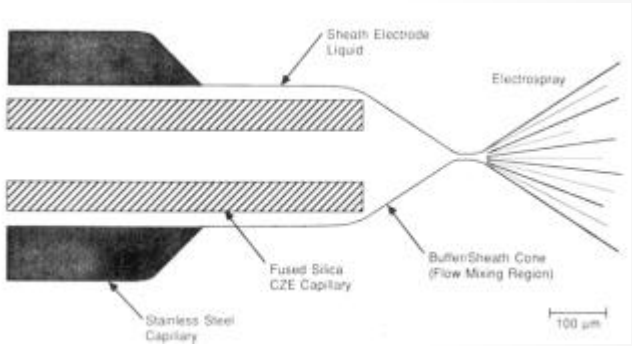
CE-ESI/MS Coupling Challenges

- No outlet vial/end electrode available when spraying into an MS.
- How to apply the field between CE capillary inlet/outlet and to MS inlet or vice-versa to obtain an electrospray and at the same time maintain a field to drive the CE.
- In CE, currents are typically 100-1000x larger than electrospray current; a safe electrical circuitery and secure ground for handling the currents and fields.
- In contrast to LC-ESI/MS, the solvent flow in CE (EOF) depends on its composition. Flow is not a settable parameter. Adapt capillary and/or BGE composition.
- BGE's with non-volatile constituents (inorganic buffers) are incompatibility with vacuum detection in MS. Eventually a BGE is selected that may or will compromise CE separation

CE-ESI/MS Coupling Retrospective

- 1988; Initial work with coaxial sheath solvent, R.D. Smith et al.*

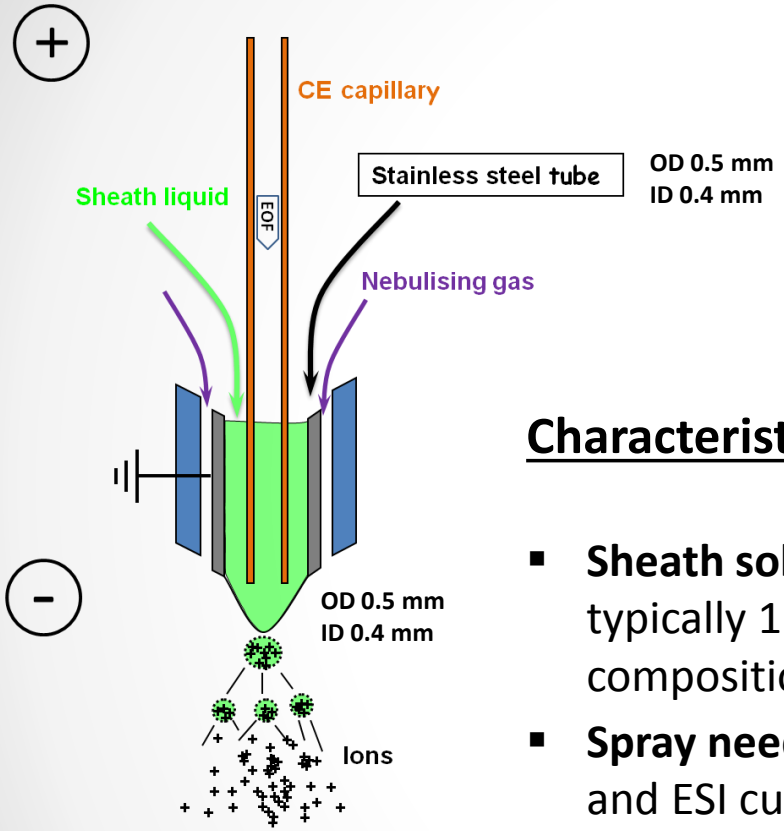




True Electrospray
Liquid electrical contact
Delivery of a sheath solvent to establish stable spray

R. D. Smith et al, Anal. Chem. 60, 436, (1988)
R.D. Smith, C.J. Barinaga, H.R. Udseth, Anal. Chem., 60, 1948 (1988)
R.D. Smith, H.R. Udseth, Nature, 331, 639 (1988).

CE-ESI/MS Coupling –Triple Tube Sprayer (Agilent)



Characteristics of "Triple Tube" Sprayer Interface

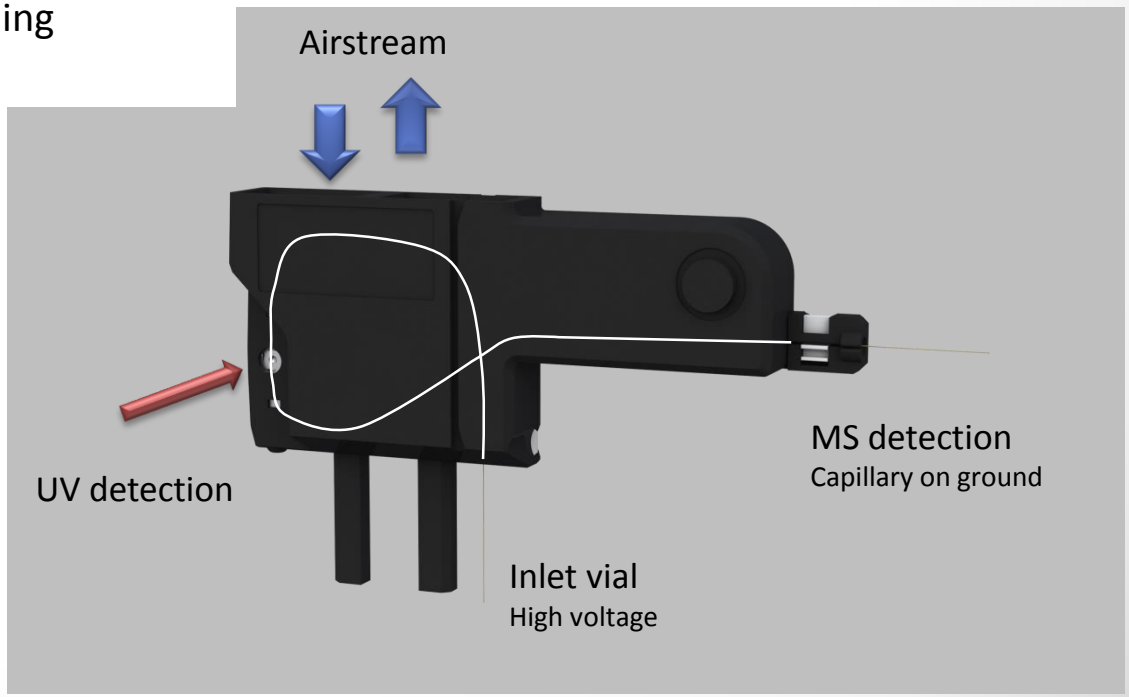
- **Sheath solvent** is added to the CE effluent at a rate of typically 1 - 10 µL/min. Spray becomes independent of BGE composition and EOF
- **Spray needle** (gray) is grounded. Common return path for CE and ESI current. ESI voltage at the MS inlet
- **Nebulizing gas** to assist spray formation
- **Sheath solvent composition** dominates electrospray ionization chemistry and may/will enhance sensitivity

Agilent Interface for CE/MS

Cassette without liquid cooling, temperature control by fast airstream providing efficient cooling and heating using a Peltier element

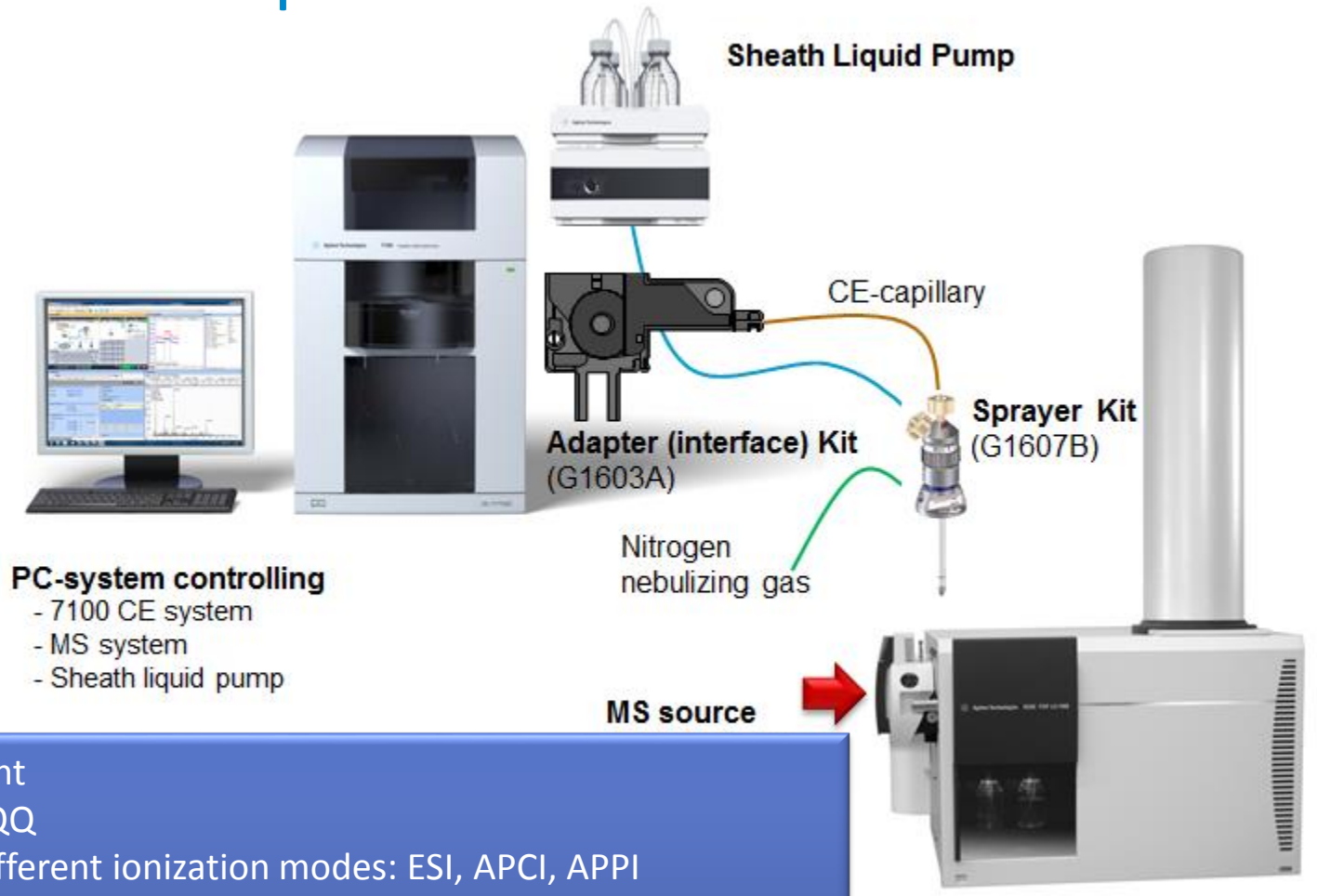
Quick change of capillaries, no sealings, no liquids no leaks.

Access to 7100 built in UV-DAD providing UV monitoring (traces and full spectra)



Slide courtesy of Agilent Technologies

Agilent CE/MS Setup



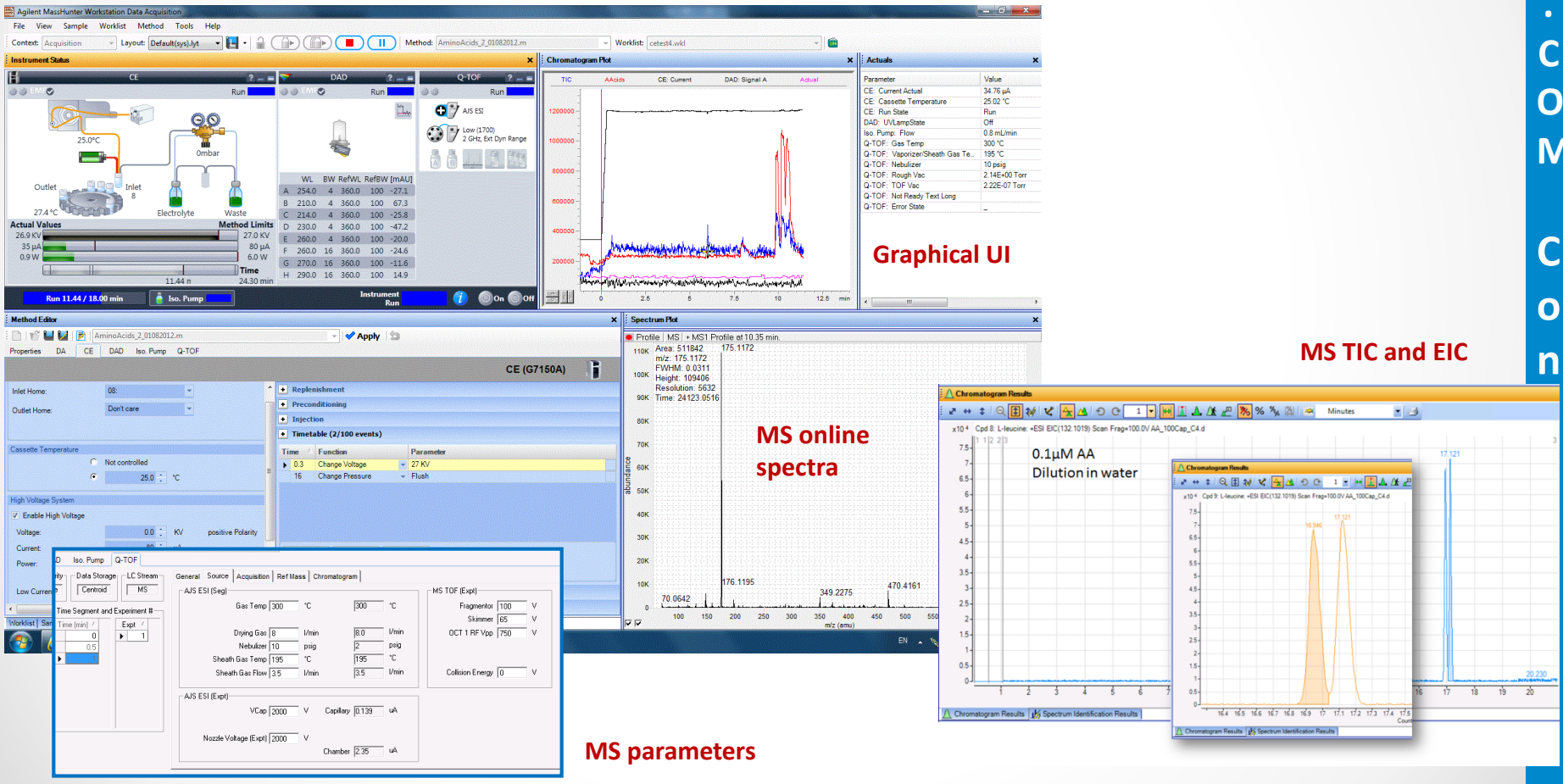
- 7100 CE instrument
- SQ, TOF, QTOF, QQQ
- Compliant with different ionization modes: ESI, APCI, APPI
- Compliant with all sources
- Orthogonal configuration (LC-MS) lets neutrals & big droplets pass
- LC pump integrated, delivering sheath solvent
- Single point SW for control, automation, data acquisition and processing

Slide courtesy of Agilent Technologies

Agilent CE/MS System

MassHunter Software for LC-MS & CE/MS

MassHunter versions B.05.01 and higher are integrating and controlling Capillary Electrophoresis for CE/MS analysis as a single software package under Windows 7 (64 bit)



Slide courtesy of Agilent Technologies

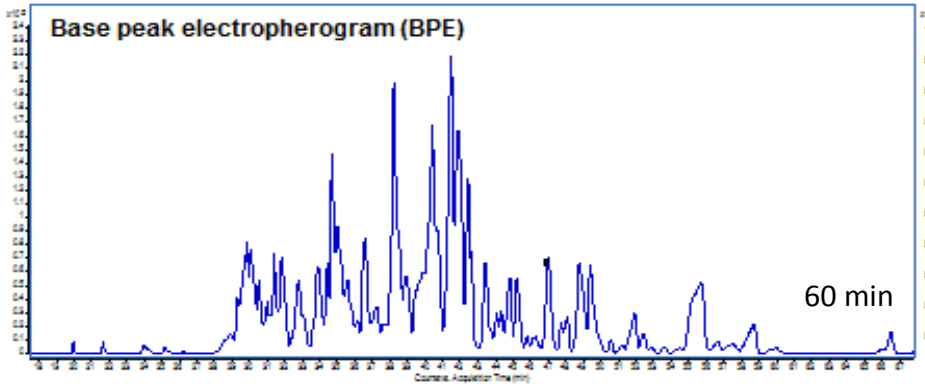
Peptide mapping: CE/MS vs. LC/MS

Comparison of Tryptic Digests

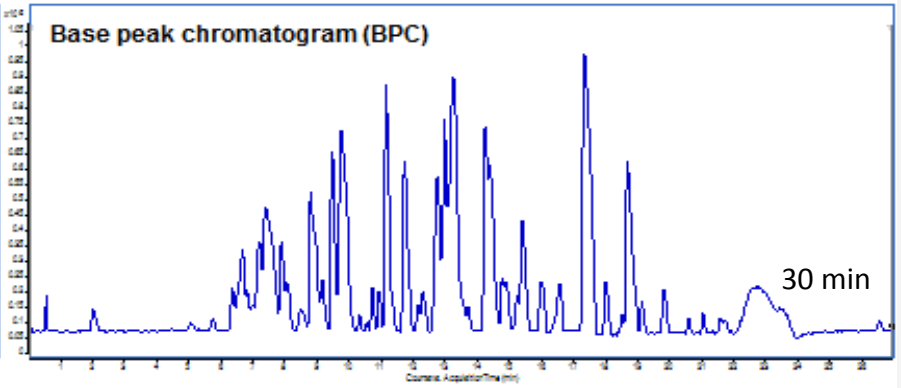
Capillary Bare fused silica, total length 85 cm, 50 µm i.d. (170 nL) Buffer 10 mM acetic acid

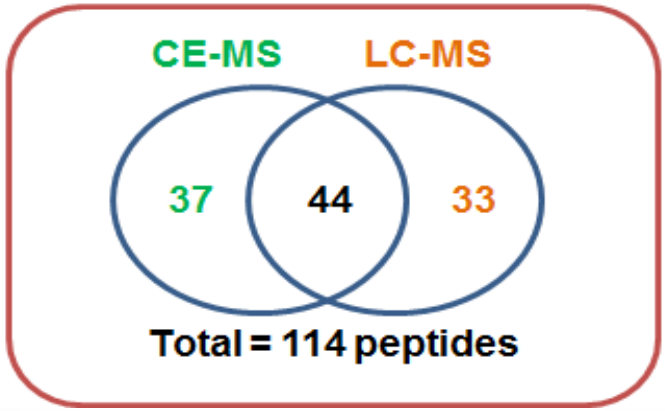
HPLC-Chip G4240-62005 , 5 µm, Agilent ZORBAX 300SB-C18, 40 nL enrichment column, a 75 µm × 43 mm analytical column (190 nL) Flow rate 0.6 µL/min. Solvents A) 0.1 % formic acid (FA) in water; B) 90 % ACN in water with 0.1 % FA

CE-QTOF MS



LC-QTOF MS





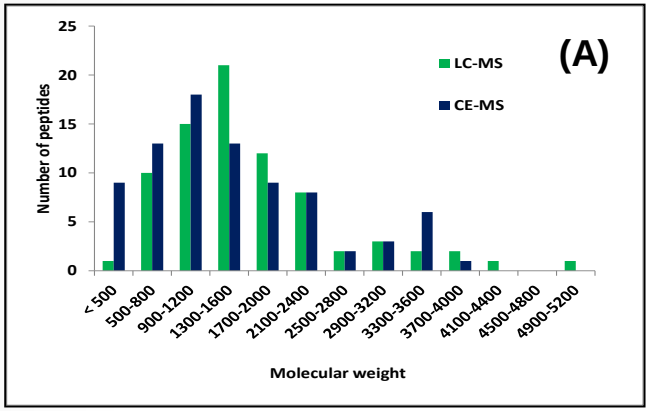
CE/MS and LC/MS peptide map of BSA

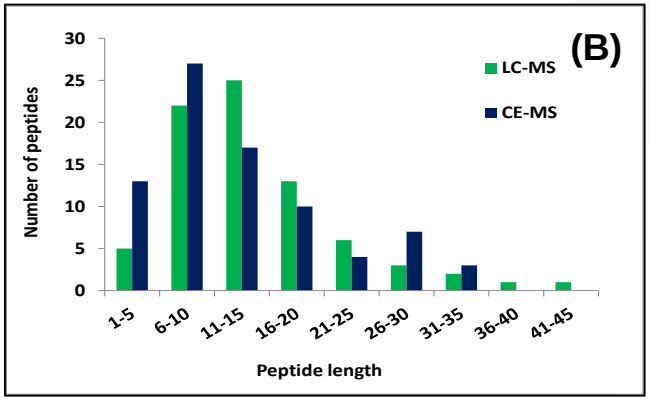
Among the total number of peptides identified (114), 37 peptides are unique to CE/MS and 33 peptides are unique to LC/MS.
Each of the techniques contained 44 common peptides.

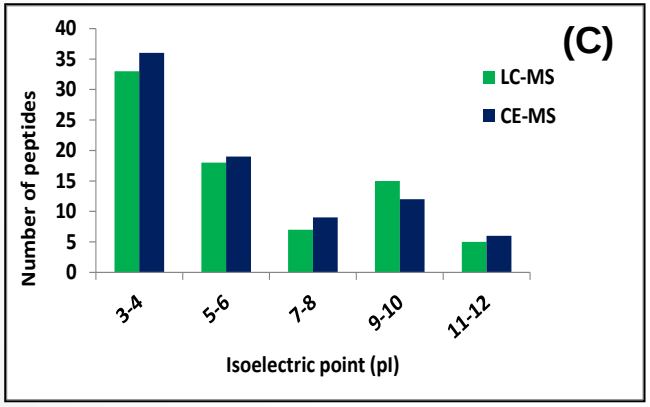
Suresh Babu CV, Agilent Application Note (Pub. Nr. 5991-2583EN)

CE/MS vs. LC/MS

Comparison of tryptic peptide maps







CE-MS and LC-MS comparison of peptide distributions.

(A) Molecular weight plot
(B) Peptide length plot
(C) Isoelectric point plot

Suresh Babu CV, Agilent Application Note (Pub. Nr. 5991-2583EN)

CE/MS vs. LC/MS

Comparison of tryptic peptide maps

	CE-QTO MS (6520)	LC-QTOF MS (Chipcube-6540)
Sample injected	44 nl (0.34pmole)= 7.7 µM in sample	2 µl (15pmole)= 7.5 µM in sample
Peptide elution window	30 min	16 min
Sequence coverage	80%	81%
Total peptides identified	82	78
Distinct peptides identified	37	33
Selectivity and resolution	change in elution order of few peptide – shows the complementary value of two techniques	
Selectivity	CE-MS is shows the best separation/ionization for hydrophilic peptides	
Peptide distribution	• Shorter peptides are represented (1-5 amino acid peptide length) • Identified peptides starting with 3 amino acid length • Low MW peptides are well presented (<500Da) • Acidic peptides (pI 3-4) are well represented	• Shorter peptides are less represented (1-5 amino acid peptide length) and also cover wide range of peptide length identified • Identified peptides starting with 4 amino acid length • Low MW peptides are less represented (<500Da)

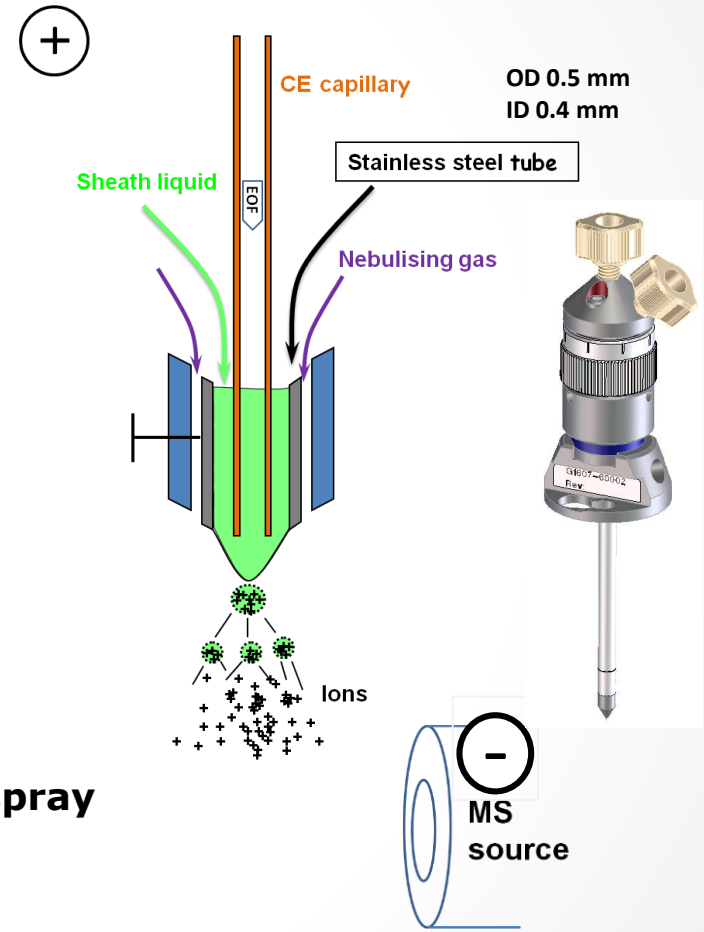
Suresh Babu CV, Agilent Application Note (Pub. Nr. 5991-2583EN)

CE-ESI/MS – Perceived Situation

Triple Tube Sprayer IF

- ☺ Since 1995 only complete commercial system for CE-ESI/MS
- ☺ Proven robustness and reliability
- ☺ Typical sensitivity 0.5 - 10 µM (in sample concentration)

But ....



Sensitivity may becomes compromised

- ☹ Solute concentration is reduced 5 - 50x by the sheath solvent depending on the actual EOF; Sensitivity will not decrease accordingly
- ☹ Because of the higher overall flow rate no nano-electrospray benefit (<100 nL/min)

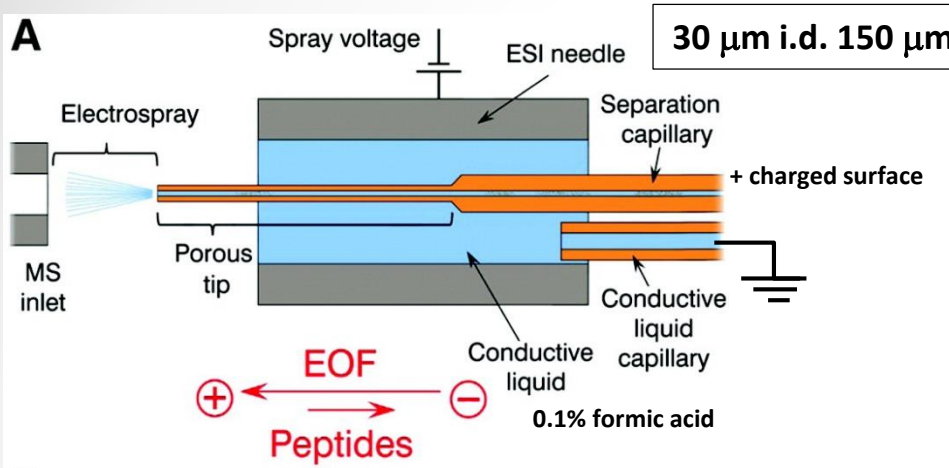
Pneumatic assistance required to establish the spray

- ☹ Undesirable hydraulic flow is observed, which need counter measures

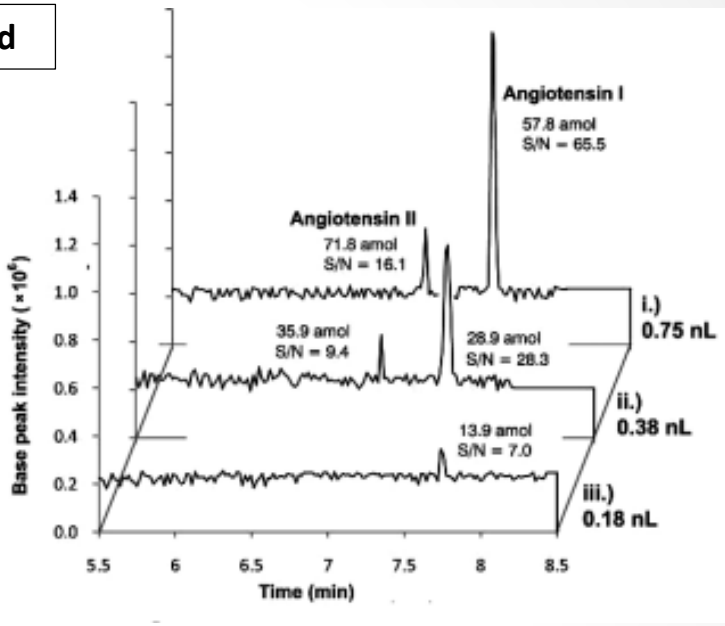
Galvanic reactions on the sprayer needle

Recent Developments in CE-MS Coupling

Porous Tip Approach (Moini, Texas A&M University)



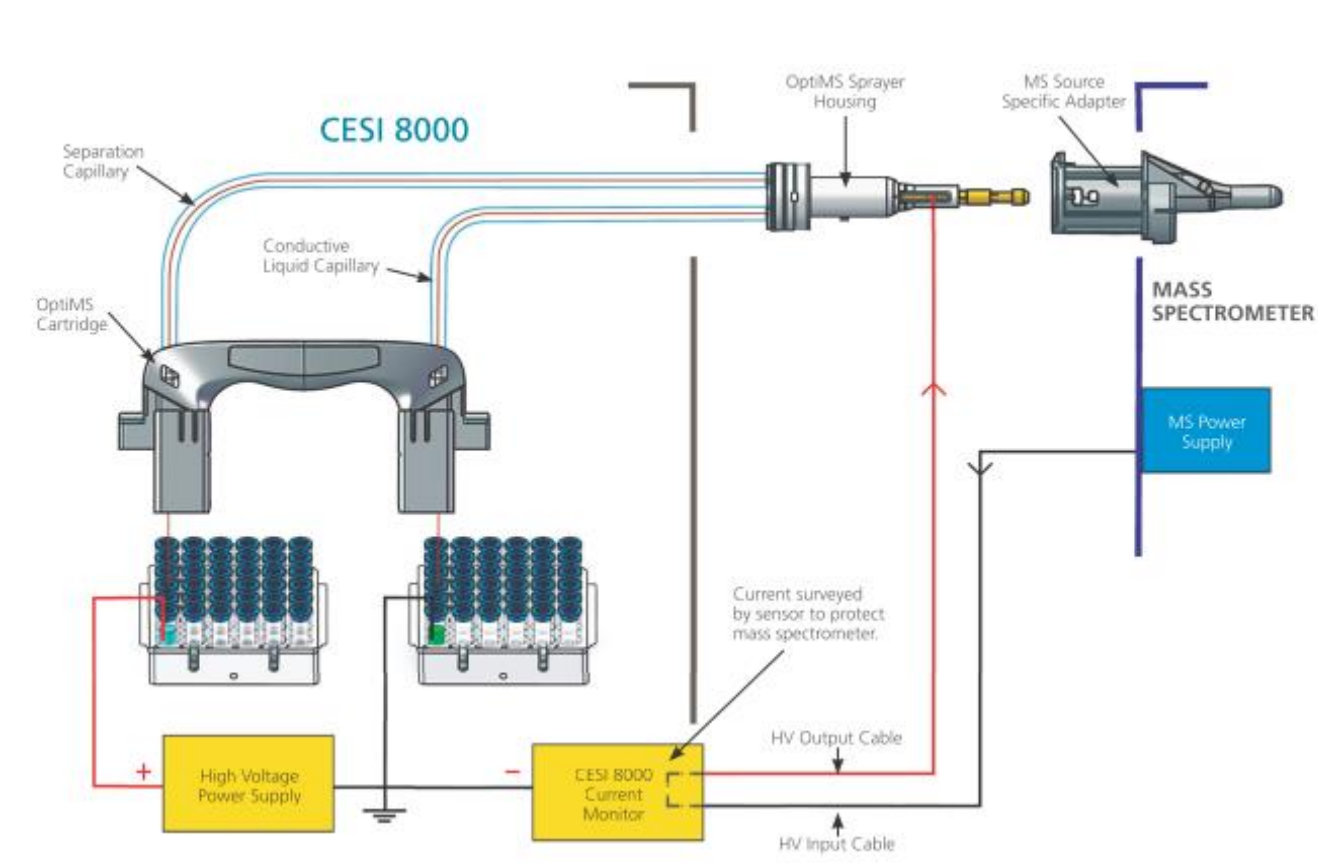
The high-sensitivity porous sprayer interface (A) schematic and (B) photograph of the prototype interface.



- Sensitivity: 10-20 nM AT1 concentration in sample
- >200 successive runs (pers. comm.)

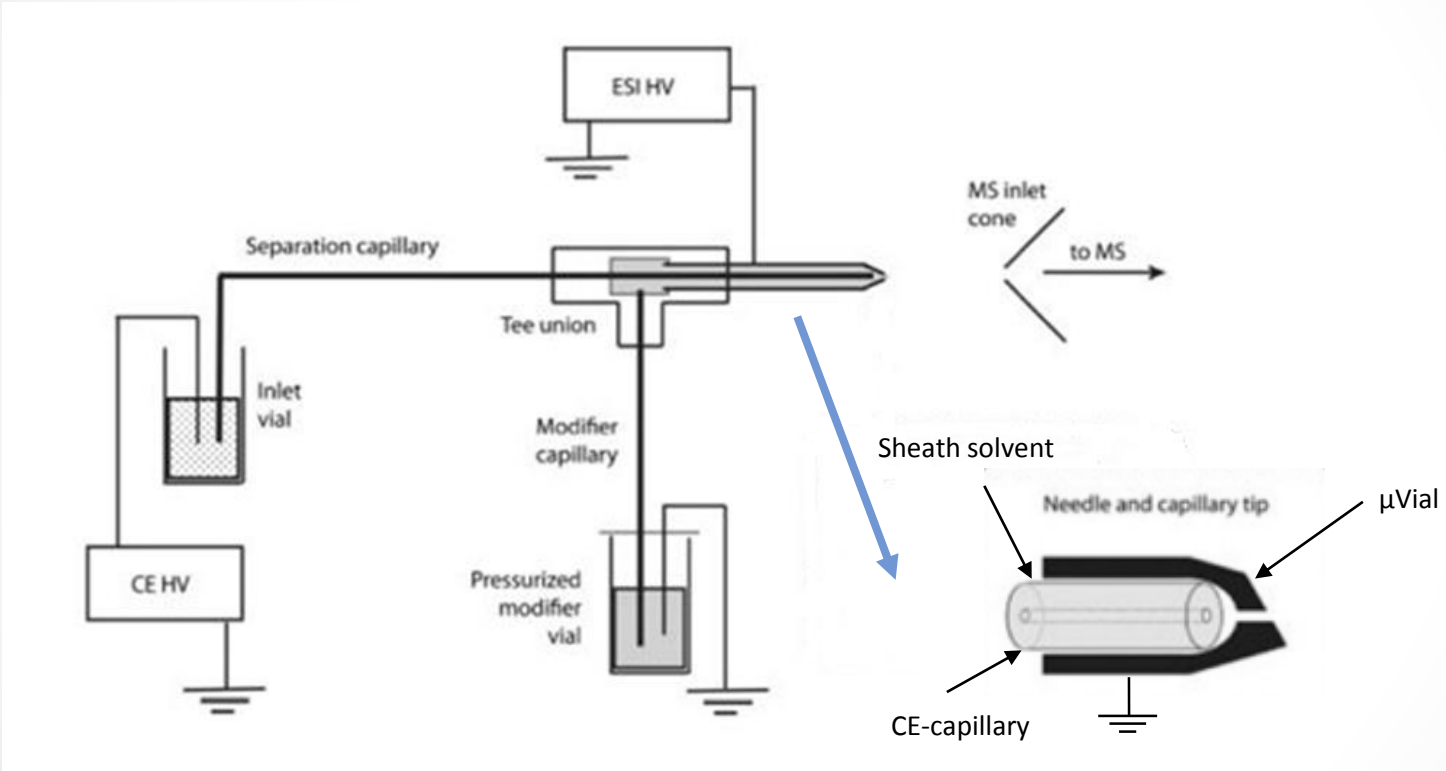
Figures taken from:
H. Lindner et al., Anal. Chem., **83**, 7297 (2011)

Sciex Separations CESI 8000



Recent Developments in CE-MS Coupling

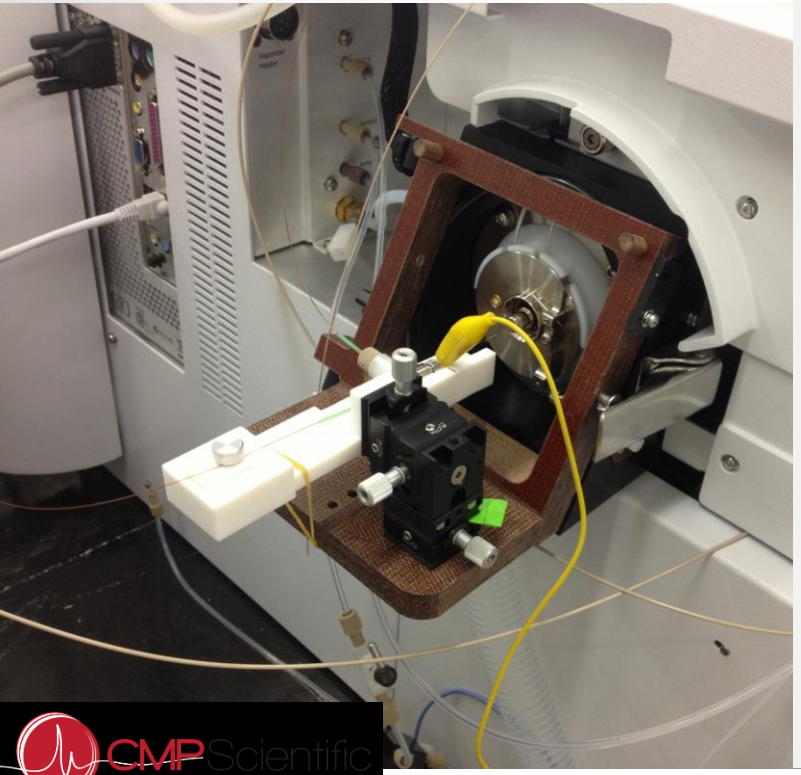
Micro Flow-Through Vial (D.D.Y. Chen et al.*, Univ. Brit. Columbia)

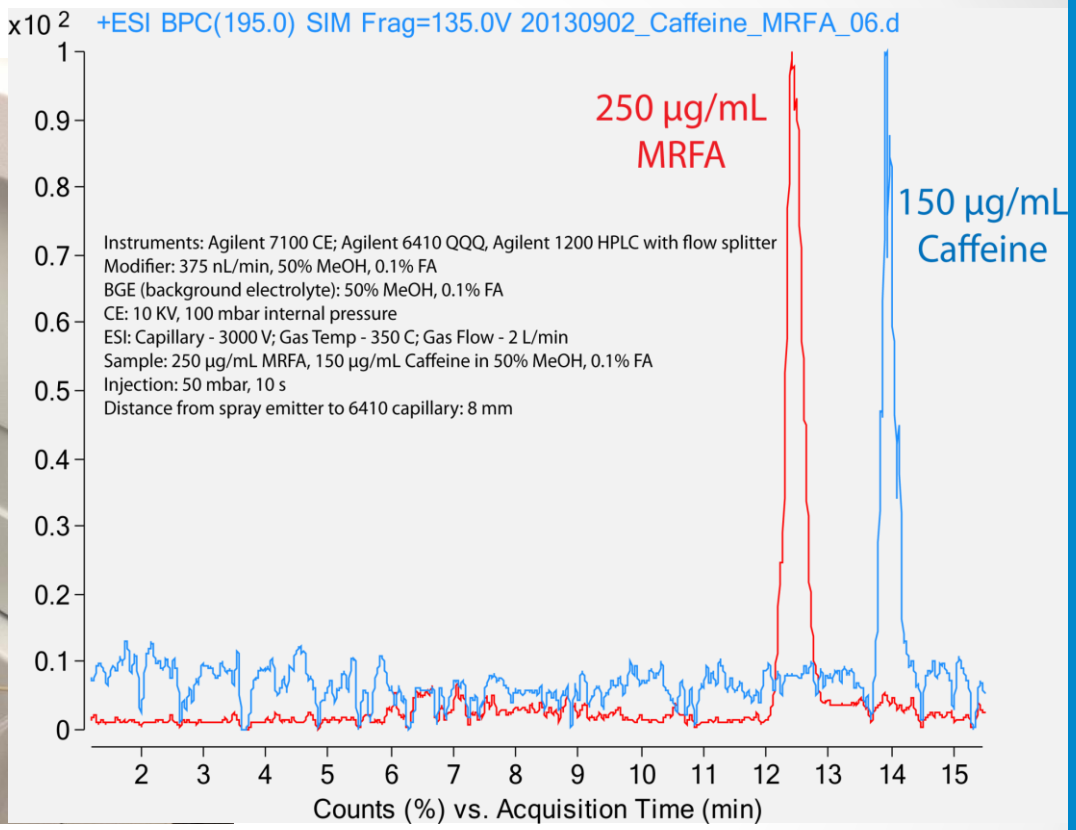


*D.D.Y. Chen et al. Anal. Chem. **83**, 4916 (2011)

Recent Developments in CE-MS Coupling

Micro Flow-Through Vial Common Ground*



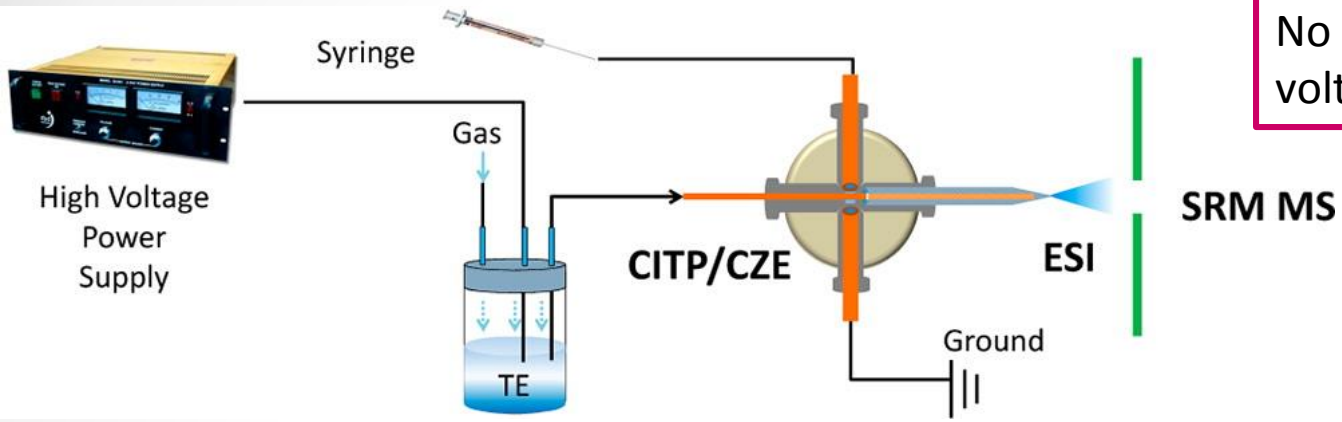


Sample: calibration mixture for electrospray ion sources (caffeine, MRFA (met-arg-phe-ala))
Sensitivity: approx. 5 µM

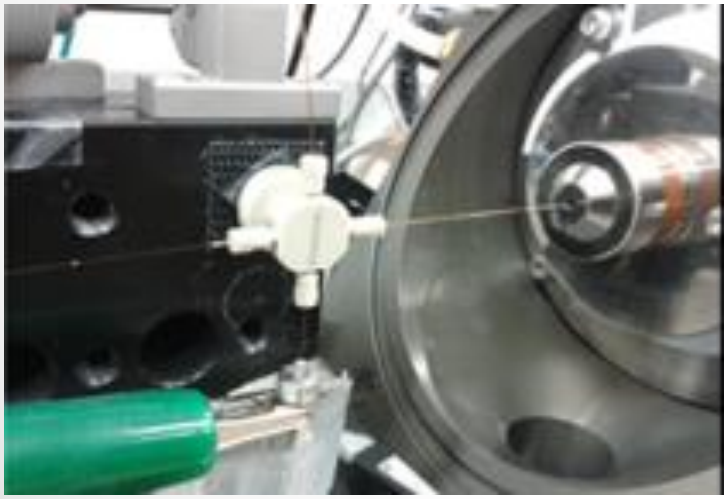
*Results and Photo courtesy of David Chen and CMP Scientific

Recent Developments in CE-MS Coupling

Sheath liquid contact approach (R.D. Smith et al.)



No additional ESI voltage used.



Separation capillary: FS 75x150 µm
Emitter capillary: FS 200x350 µm, end etched with HF and orifice 50 µm
BGE: 25 mM ammonium acetate, pH 4
Sheath solvent and TE:9/1 0.1 M acetic acid/methanol

Sample: short peptides in BSA digest

Sensitivity: 50 pM with CITP sample pre concentration

*R.D. Smith et al., Anal. Chem., **84**, 10395 (2012) and Chenchen Wang et al, Poster presented at MSB2013, Charlottesville

Is there a future for CE-MS?

- Achieving highest sensitivity remains top objective; but…
  - unlike HPLC, CE has limited sample volume loading capacity and cannot be scaled like HPLC.
  - in contrast to HPLC with SPE pre-concentration, sweeping or cITP pre-concentration methods are required and regarded “difficult”.
- Fact is ….
  - given the same amount entered into the MS, CE results in higher peaks than HPLC!
  - the premier user’s interest though is the analyte concentration in the sample
  - therefore, CE-MS will be preferred method for measurement of very low concentration polar/charged analytes in very small sample volume
- Conventional coaxial solvent sheath flow IF pairs adequate sensitivity (with up-to-date MS) with ease of use and robustness
- Porous tip and μVial-flow through IF seem a promising pathway towards CE-ESI/MS and are being commercialized.
- Commercialization (affordable) will be the key for success of new sheathless CE-ESI/MS coupling methods

More Information

- PDF-copy of this presentation can be found at http://www.rozing.com (registration required!)
- Agilent Primer on CE at: http://www.chem.agilent.com/Library/primers/Public/5990_3777EN.pdf
- Agilent Primer on CIEF at: http://www.chem.agilent.com/Library/primers/Public/5991-1660EN.pdf