

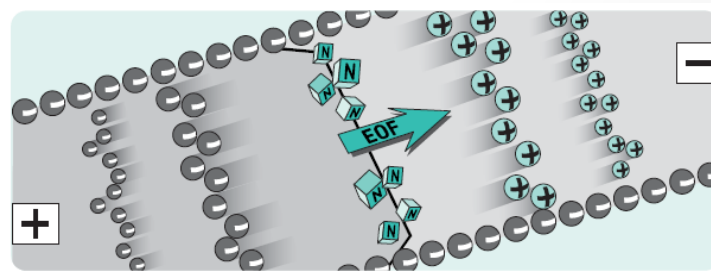
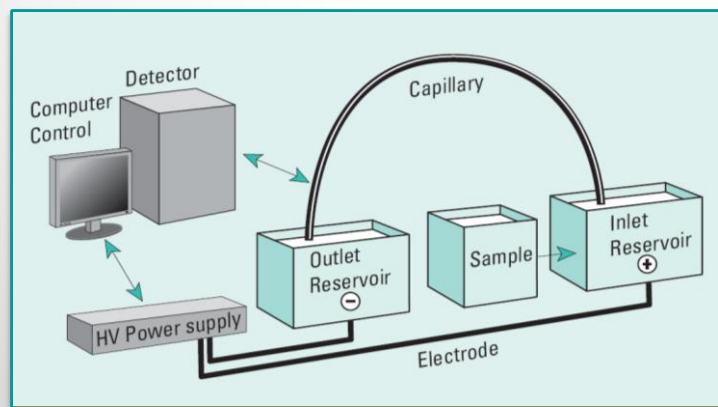
# Capillary Electrophoresis (CE) and CE-MS

Taken in part from “Primer on Capillary Electrophoresis”,  
Agilent Technologies Pub. Number 5990-3777EN

# Capillary Electrophoresis

**Ideally for ionizable/charged analytes**

- Fast analysis of charged substances like biomolecules, small basic or acidic drugs and ions
- Separation based on compound mobility (mass/charge) in an electrical field
- Very high resolution separations (especially HMW substances)
- Takes ultra small sample volumes (few nL)
- Less sample prep required (in principle no stationary phase, just an open fused silica capillary tube)
- Orthogonal technique to HPLC → complementary information
- Low consumption of sample and buffer (green method)



# Capillary Electrophoresis

## Principle

- Separation is based on the differences in solute velocity in an electric field
- The electrophoretic mobility of a compound is a molecule specific property (depending on molecule mass and charge)

$$v = \mu_e E$$

where  $v$  = ion velocity  
 $\mu_e$  = electrophoretic mobility  
 $E$  = applied electric field

### Chromatography:

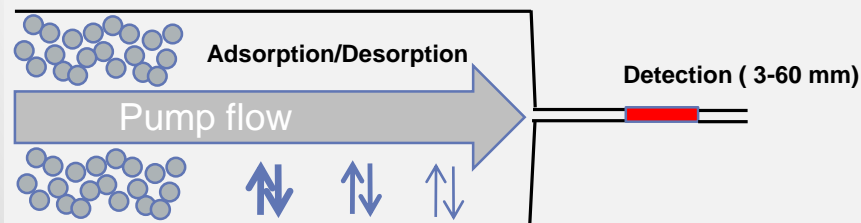
Solute velocity: constant

Flow rate: nl-ml/min

Columns: particulate adsorption material

Separation: difference in solute adsorption/desorption

Peak width: depending on  $d_p$ ,  $u_o$ ,  $D_i$



### Electrophoresis:

Solute velocity: a) EOF pH dependent (nL/min)

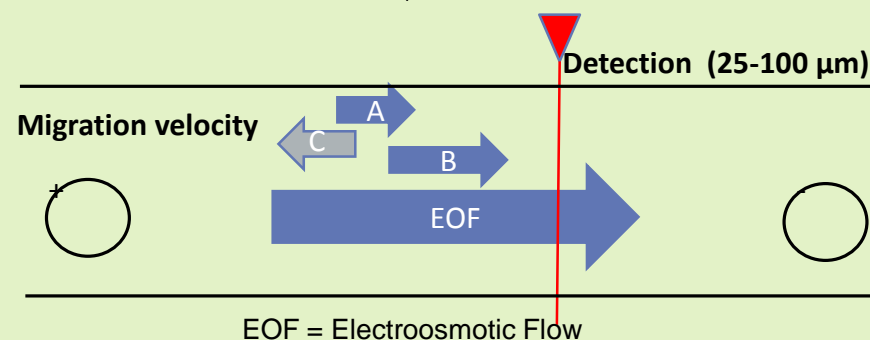
b) mobility

Flow rate nl/min

Columns: Fused silica capillaries

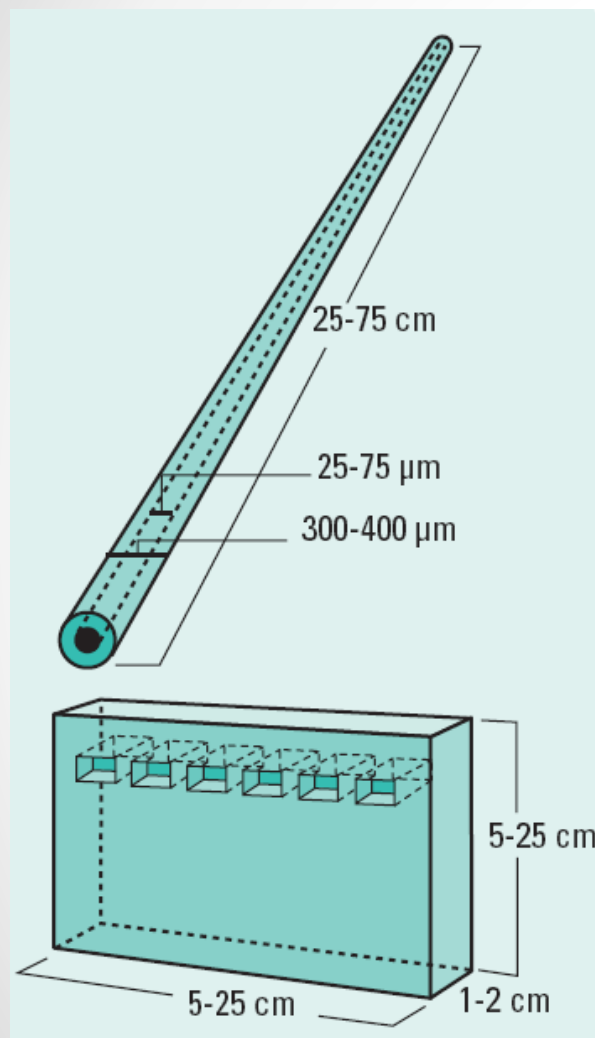
Separation: Differential solute speed / moving direction

Peak width: depending  $D_i$



# Capillary Electrophoresis

## Principle



## Historical background, current status and applications of CE

Electrophoresis as a separation technique was introduced by Tiselius in 1937. For this work he was awarded a Nobel Prize.

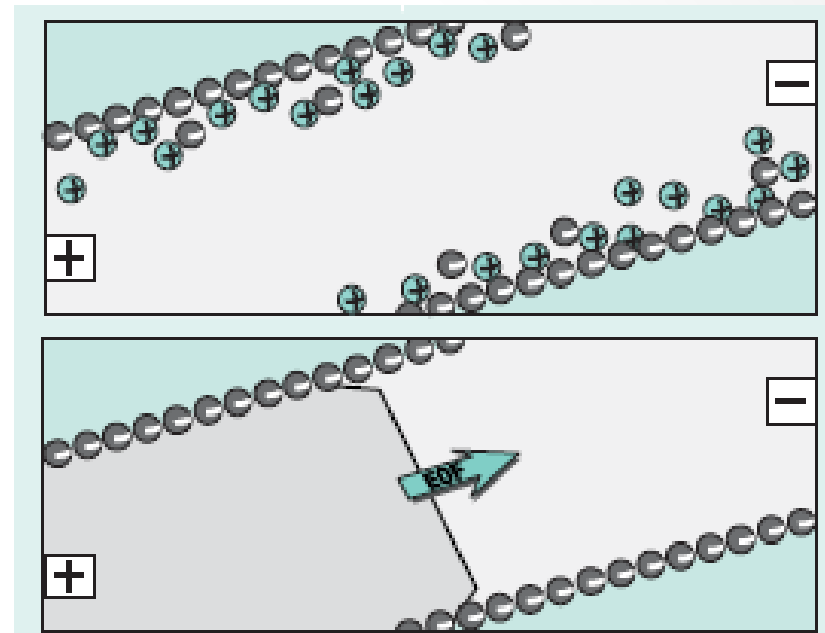
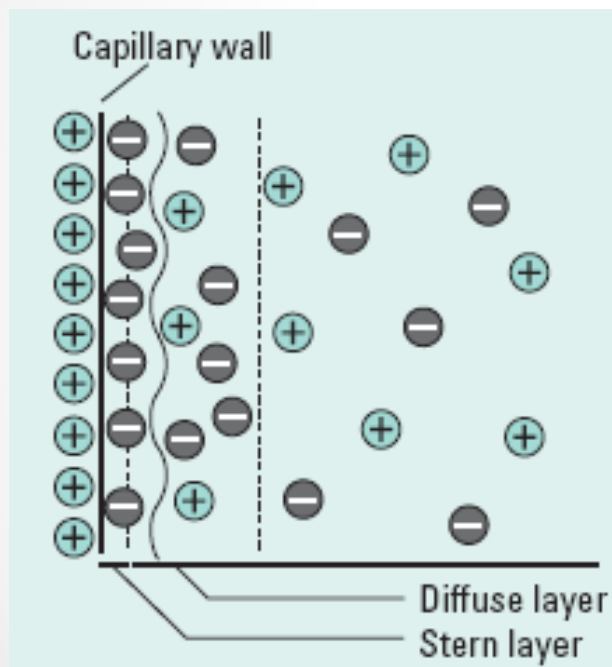
Separation efficiency in free solution - limited by thermal diffusion and convection → gels to obstruct convection (slab gel electrophoresis)

In the early 1980s Jorgenson and Lukacs advanced the technique by using 75- $\mu\text{m}$  i.d. fused silica capillaries. Jorgenson also clarified the theory, described the relationships between operational parameters and separation quality, and demonstrated the potential of capillary electrophoresis (CE) as an analytical technique.

# Capillary Electrophoresis

## Electro-osmotic flow

### Electro-osmotic flow (EOF)



$$v_{\text{EOF}} = (\epsilon \zeta / \eta) E$$

or

$$\mu_{\text{EOF}} = (\epsilon \zeta / \eta)$$

where:

$v_{\text{EOF}}$  = velocity

$\mu_{\text{EOF}}$  = EOF "mobility"

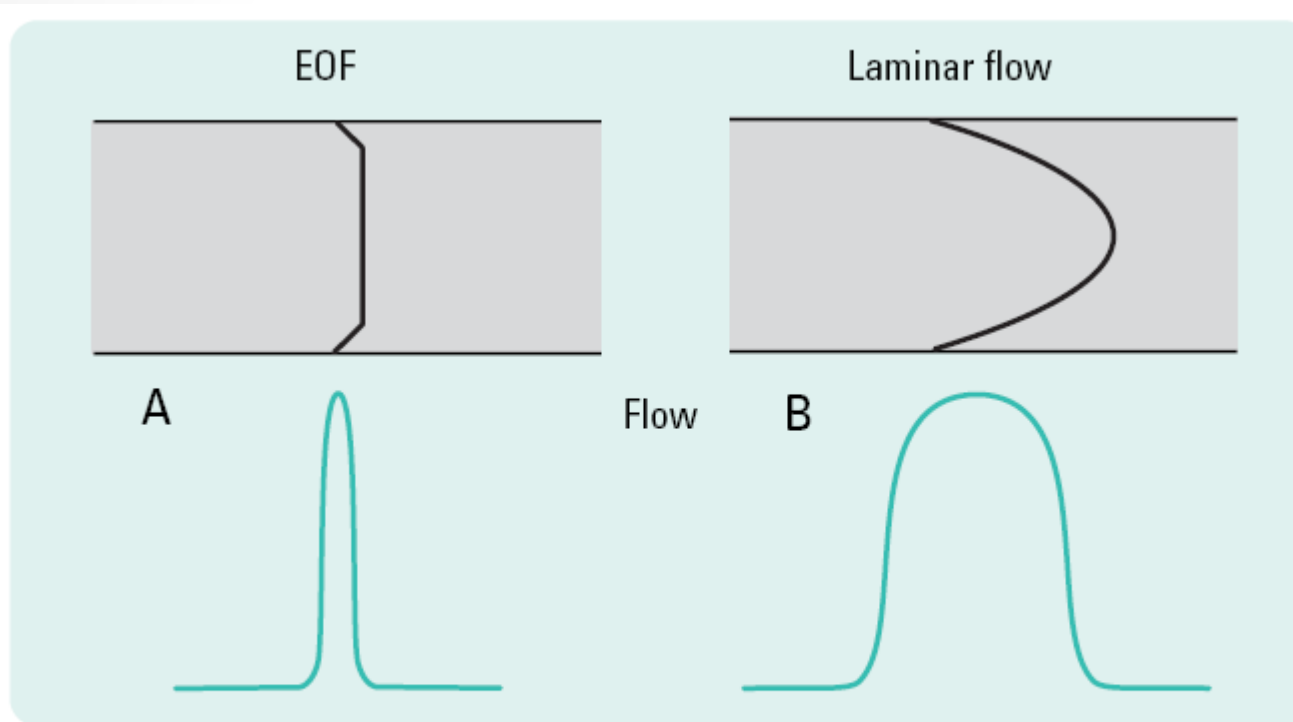
$\zeta$  = zeta potential

$\epsilon$  = dielectric constant.

# Capillary Electrophoresis – basics

## Electro-osmotic flow

Velocity profile of EOF is flat in contrast to the parabolic velocity profile in pressure driven flow in capillaries



**Figure 1.7 A, B**  
Flow velocity profiles and corresponding solute zones in electro and pressure driven flow.

# Capillary Electrophoresis

## Control of Electro-osmotic Flow

The Electro Osmotic Flow **EOF**, can be beneficial in moving neutrals and even molecules with opposite charge (anions) to the detector at the cathode.

Sometimes it is better to avoid internal flow rates by EOF to depend solely on the mobility of charged compounds.

Variable	Result	Comment (see subsequent sections)
<b>Electric field</b>	EOF changes proportionally with electrical field	High field generates more heat in the capillary. Eventually short-circuits and sparking.
<b>Buffer pH</b>	EOF decreases at low pH and increases at high pH	May change solute charge (figure 1.6)
<b>Ionic strength or buffer concentration</b>	EOF increases at low ion strength	High ionic strength generates high current causing Joule heating Lower buffer capacity at low ion strength; possible sample adsorption; limits sample stacking (see chapter 3) Peak shape distortion if conductivity of the electrolyte differs from sample conductivity
<b>Temperature</b>	EOF changes due to viscosity change (2 – 3%/°C)	Capillary temperature must be controlled
<b>Organic modifier to the electrolyte</b>	Changes zeta potential and electrolyte viscosity	Complex changes of EOF; effect most easily determined experimentally. May alter selectivity of separation
<b>Additives to the electrolyte (e.g. surfactants)</b>	Change magnitude and direction of EOF s. Anionic surfactants can increase EOF; Cationic surfactants can decrease EOF	Dynamical adsorption to capillary wall via hydrophobic and/or ionic interaction
<b>Neutral hydrophilic polymer</b>	Decreases but controls EOF by shielding surface charge and increasing viscosity	Adsorbs to capillary wall via hydrophilic interactions. Suppresses solute/wall interactions
<b>Covalent bonded surface coating</b>	EOF changes depending on the charge and polarity of the coating	Many modifications possible (hydrophilicity or charge) Stability can be problematic. Changes surface properties and therefore solute/surface interactions possible

# Capillary Electrophoresis

## Modes of Operation – Compare with HPLC

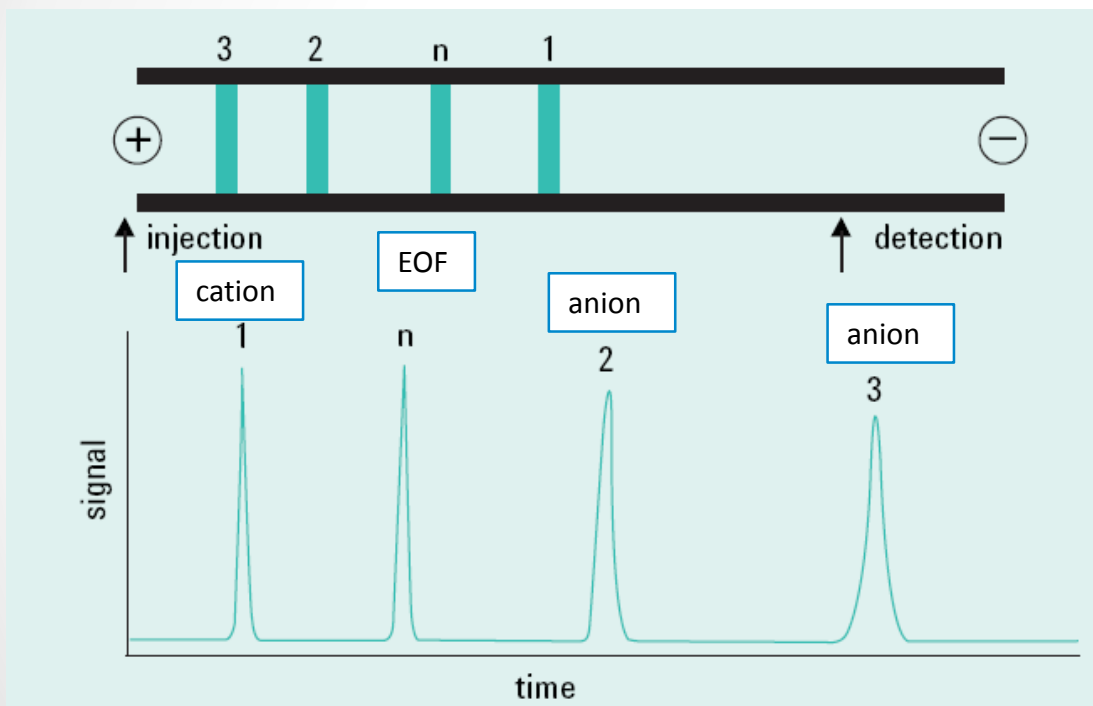
Liquid Chromatography	Electrophoresis
<b>Elution:</b> Separation based on partition between a mobile and stationary phase. Reversed Phase, Ion Exchange, Normal & Polar Bonded Phase, Hydrophobic Interaction	<b>Elution:</b> Separation based on differences of zone velocity. Capillary Zone Electrophoresis CZE, Micellar Electrokinetic Chromatography Chromatography Capillary Electrochromatography, CEC
<b>Molecular Sieving:</b> Separation based on movement through a stationary phase according to size and shape. Size Exclusion Chromatography	<b>Molecular Sieving:</b> Separation based on movement through a stationary or immobile phase (gel) according to size and shape. Capillary Gel Electrophoresis, CGE
<b>Chromatofocussing:</b> Separation based on pH gradient delivered by mobile phase through an ion exchange stationary phase	<b>Capillary Iso-Electric Focussing, CIEF:</b> Separation by movement through a stationary pH gradient in the run buffer
<b>Displacement Chromatography:</b> Separation based on moving a strong adsorbing zone (displacer) through a stationary phase. Sample molecules move as zones in front of the displacer with the solvent velocity	<b>Capillary Iso-Tachopheresis, CITP:</b> Separation based on a moving electric field gradient from leading to terminating electrolyte causing all sample molecules to move as connected zones with constant concentration with the same velocity



# Capillary Electrophoresis

## Modes of Operation

### Capillary Zone Electrophoresis (CZE)



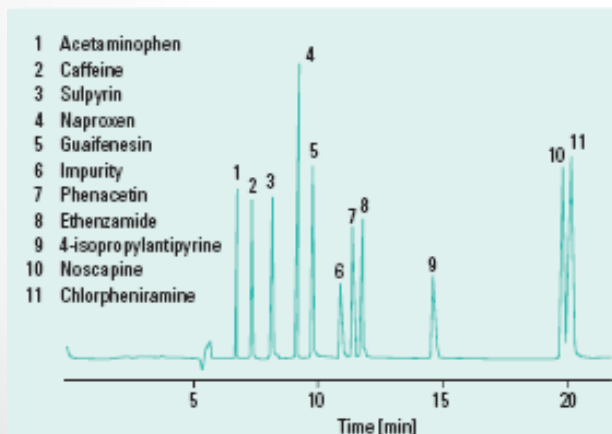
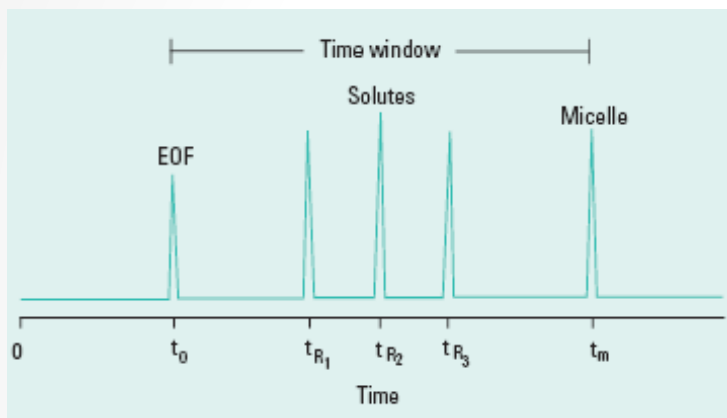
Charged molecules migrate driven by the electrical field. The larger the charge/size ratio the faster it moves.

Due to fixed charges in the fused silica capillary wall and mobile counter ions in the buffer an overall flow, the Electro Osmotic Flow **EOF**, builds up (without any mechanical pump) that drives neutral molecules towards the detector (here at the cathode).

Wall coating (permanent or dynamic, SMIL) is used to suppress, control or modulate the magnitude and direction of the EOF.

# Capillary Electrophoresis - modes

## Micellar Electro-Kinetic Chromatography (MEKC)



	Biological detergents	CMC (mM)	Aggregation number
Anionic	SDS	8.2	62
Cationic	DTAB	14	50
	CTAB	1.3	78
Non Ionic	Octylglucoside	—	—
	n-Dodecyl- $\beta$ -D-maltoside	0.16	—
	Triton X-100	0.24	140
Zwitterionic	CHAPS	8	10
	CHAPSO	8	11
Bile Salt	Cholic acid	14	2–4
	Deoxycholic acid	5	4–10
	Taurocholic acid	10–15	4

MEKC is the method of choice to separate neutral molecules by CE. The molecules partition between the lipophilic micelles and the aqueous buffers

# Capillary Electrophoresis

## Modes of Operation

### Capillary Electrochromatography (CEC)

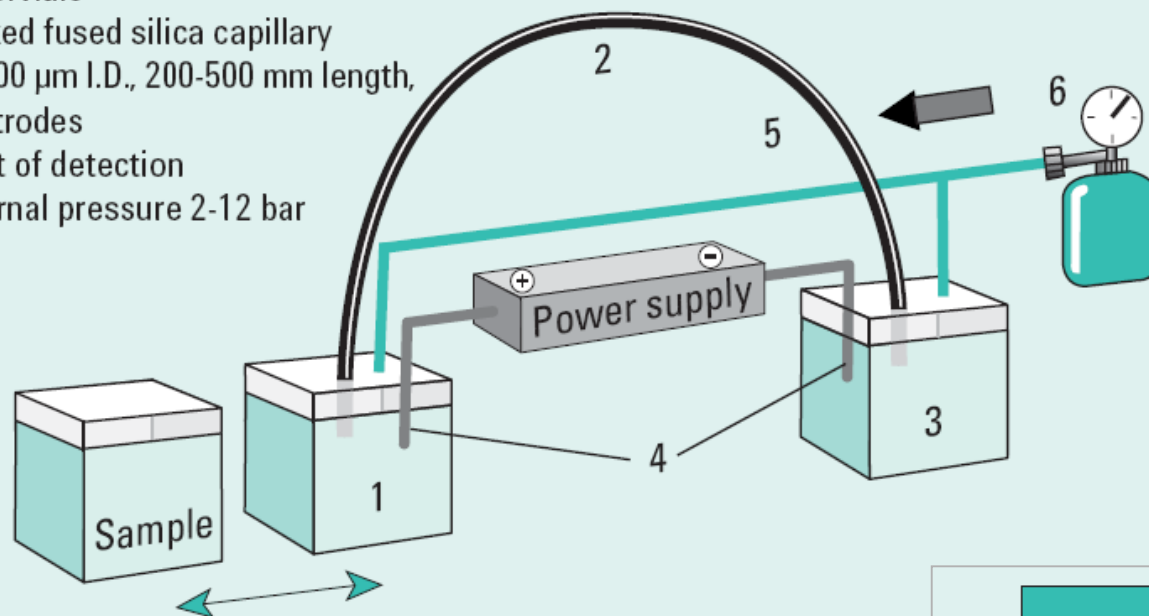
1, 3 buffervials

2 packed fused silica capillary  
50-200  $\mu\text{m}$  I.D., 200-500 mm length,

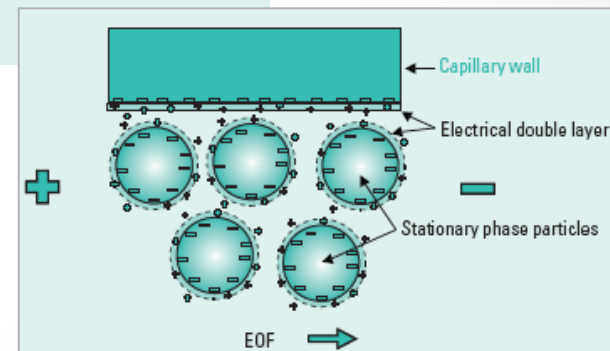
4 electrodes

5 point of detection

6 external pressure 2-12 bar



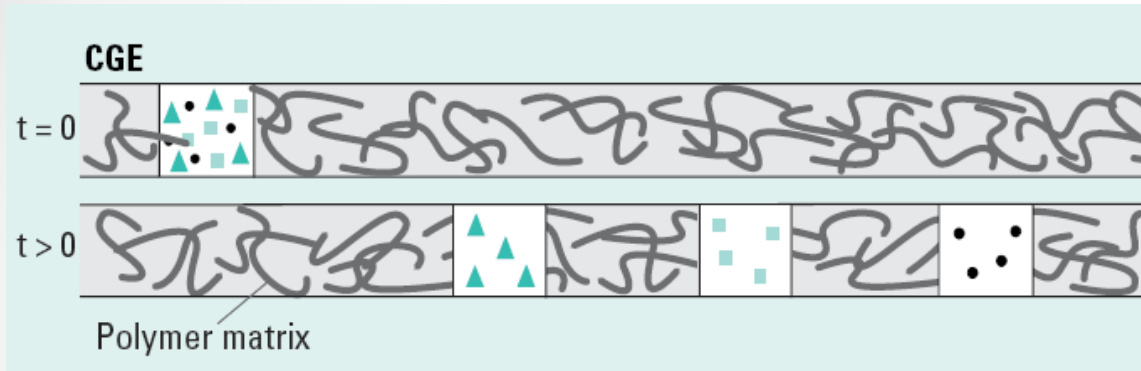
CEC is a mixture of Electrophoresis and Chromatography within capillaries packed with C18 or other HPLC materials. The EOF works as pumping system to move the liquid through the column.



# Capillary Electrophoresis

## Modes of Operation

### Capillary Gel Electrophoresis (CGE)



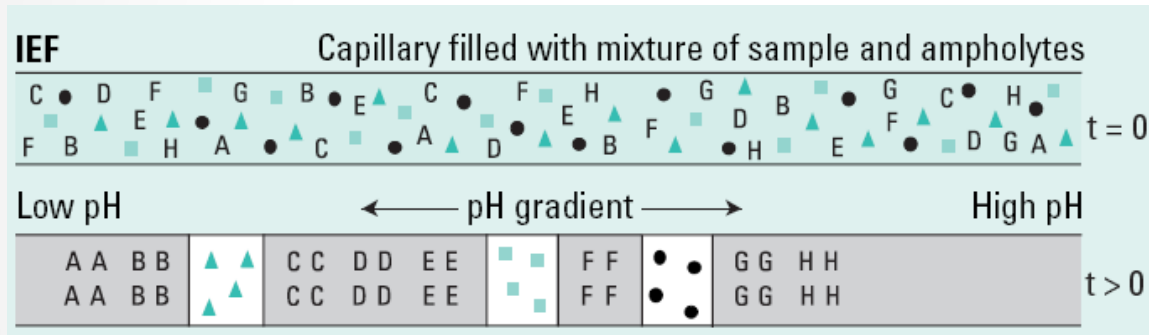
Polymer	Concentration	Application
Crosslinked polymers		
Polyacrylamide/bis- acrylamide	2–6 % T, 3–6 % C <sub>0</sub>	Oligonucleotides, DNA sequencing, native and SDS-bound proteins
Linear polymers		
Polyacrylamide	< 0.1–6 %	Restriction fragments
Hydroxylalkylcellulose, polyvinylalcohol, dextran	6–15 %	Oligonucleotides, DNA sequencing, proteins
Agarose	0.05–1.2 %	Restriction fragments, proteins

CGE is used to separate biopolymers (SDS-proteins, oligonucleotides, DNA, RNA). Since the mass to charge ratio of stays the same their mobilities are equal. To separate a sieving medium is needed to separate larger from smaller (faster moving) compounds

# Capillary Electrophoresis

## Modes of Operation

### Capillary Isoelectric Focusing (CIEF)



CIEF separates proteins and peptides by their different pI values. A pH-gradient allows molecules like proteins only to migrate until their pI equals the pH of the gradient. There the molecule is neutralized and can't move further – it is focused by the field.

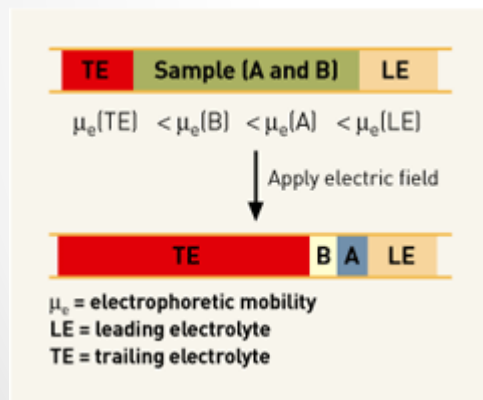
See also Agilent Technologies Pub. Number, 5991-1660EN

# Capillary Electrophoresis

## Modes of Operation

### Capillary Isotachopheresis (CITP)

#### ITP

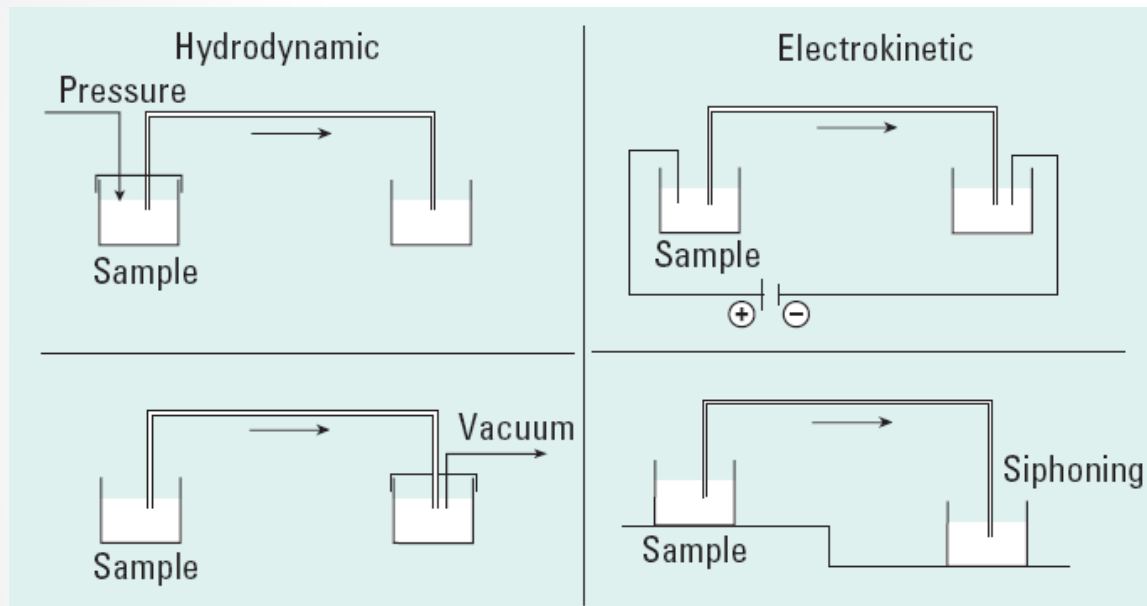


ITP is often used for „stacking“ procedures in CE to pre-concentrate compounds at boundaries of different conductivities which can increase detectability substantially.

# Capillary Electrophoresis

## Instrumental Aspects

### Sample introduction



# Capillary Electrophoresis

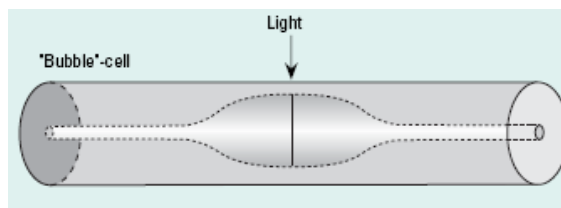
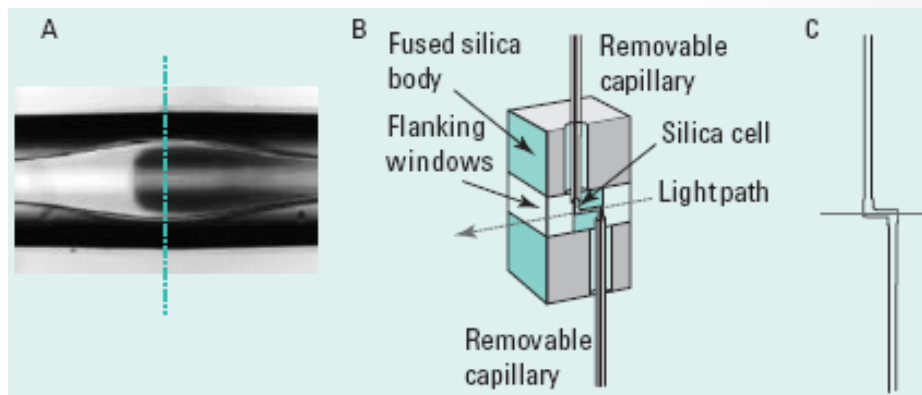
## Instrumental Aspects

### Designs of flow cells

UV detection is based on the Lambert-Beer law that states that sensitivity is directly proportional to the length of light path within a detector cell. In CE detection happens through the capillary where inner diameters are usually between 25 and 75  $\mu\text{m}$ .

This limitation was tackled by HP and later Agilent in developing extended light path capillaries for CE offering 3x or 5x the diameter of a standard capillary.

Another way was the development of the HighSensitivity Cell offering a Z-shaped cell with 1.2 mm path length and a volume of 12 nL

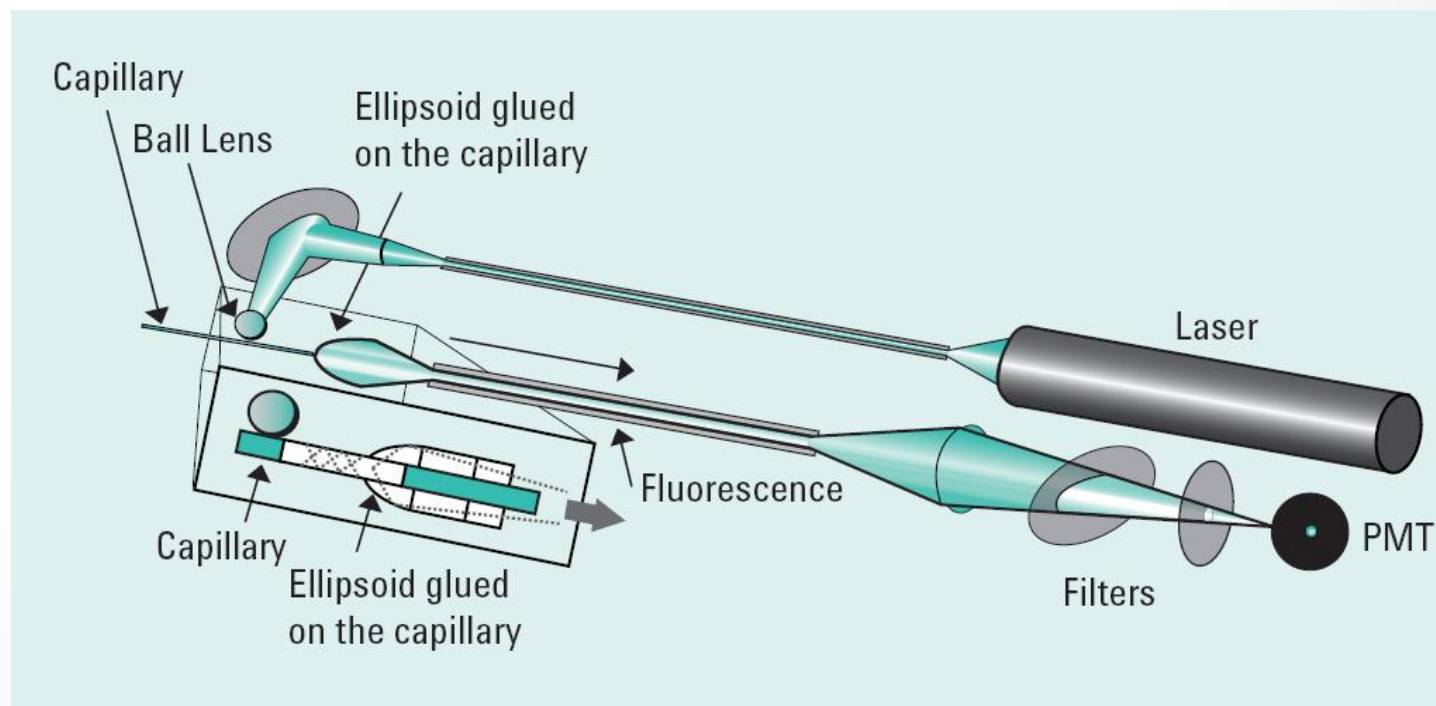




# Capillary Electrophoresis

## Instrumental Aspects

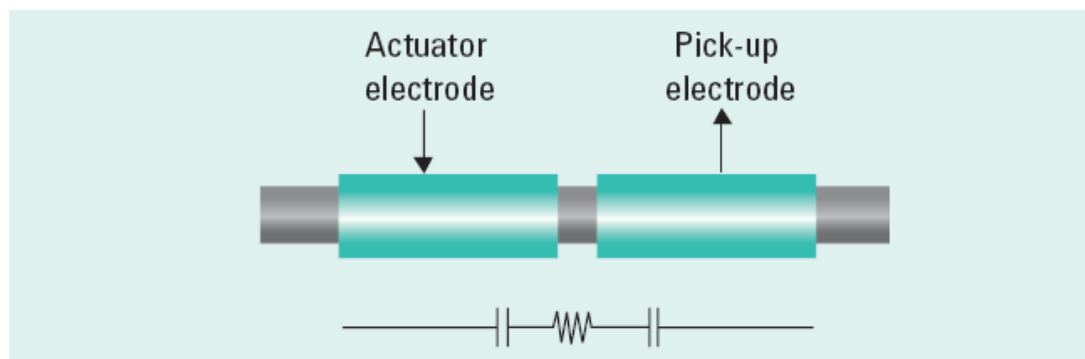
### Laser Induced Fluorescence (LIF) Picometrics



# Capillary Electrophoresis

## Instrumental Aspects

### Contactless Conductivity Detection (CCD) TraceTec (Innovative Sensor Technologies)

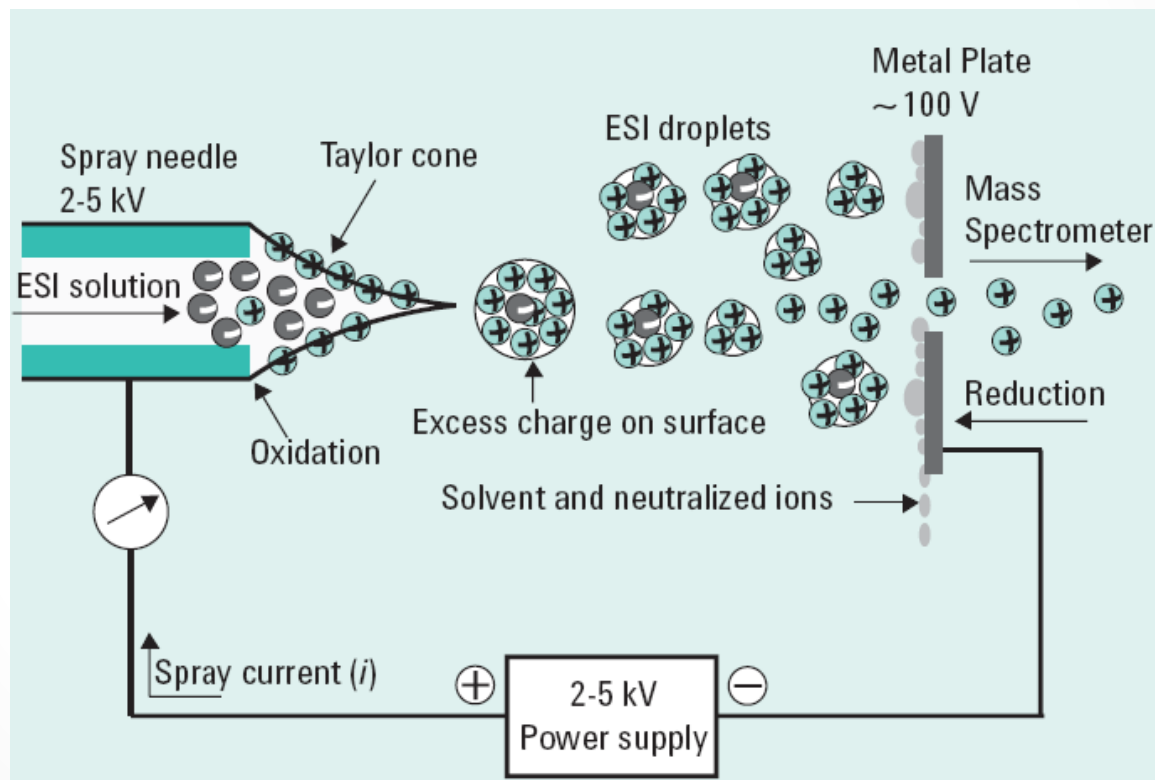


CCD offers another way to detect ions besides the indirect UV methods.

# Capillary Electrophoresis - technical

## Capillary Electrophoresis Coupled with Mass Spectrometric Detection

### Electrospray-MS

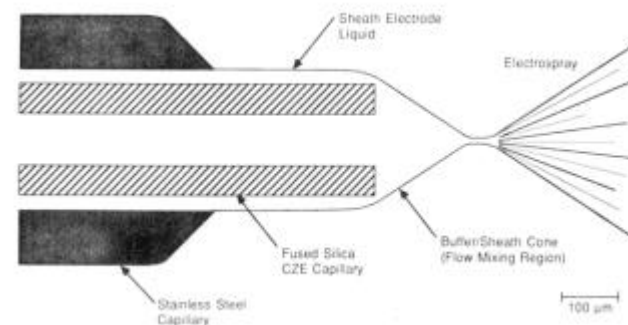
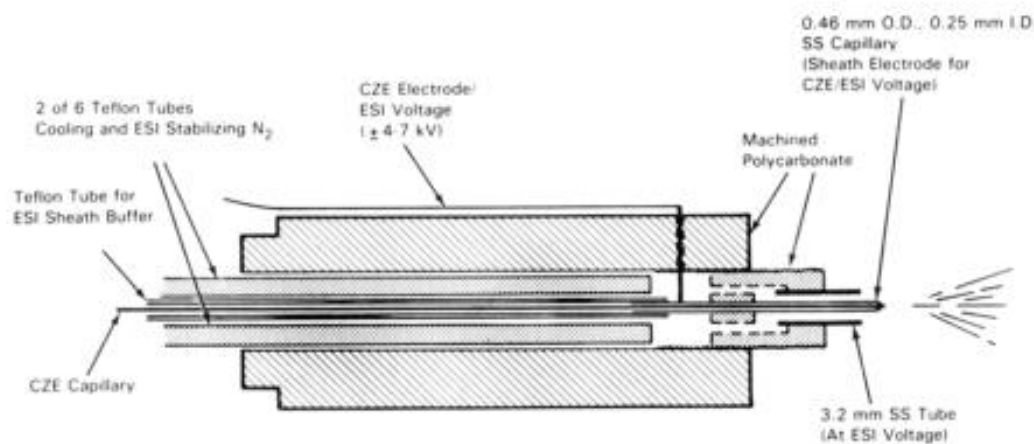


# Main challenges for CE-ESI/MS:

- No outlet vial/end electrode available when spraying into an MS
- How to apply the field between CE capillary exit and 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 circuit and secure ground for handling the currents and fields and protect the MS system
- In contrast with HPLC-ESI/MS, the solvent flow in CE, i.e. the EOF depends on its composition. This may impair the optimization of CE separation
- Like in HPLC; incompatibility of BGE's with non-volatile constituents and 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).

**ROZING • COM**  
**C o n s u l t i n g**

- ROZING • COM**  
**C o n s u l t i n g**



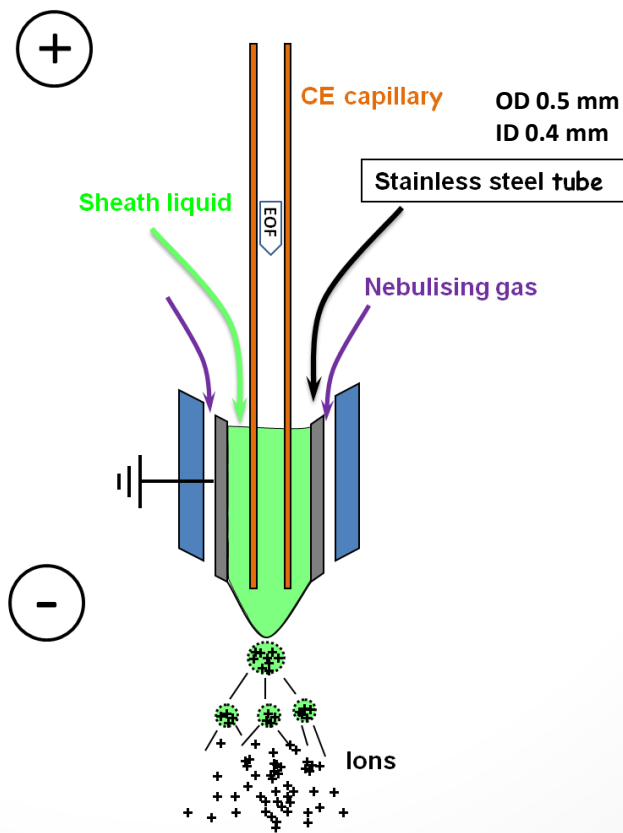
# CE-ESI/MS Coupling Retrospective

Since 1995:

- In practice, skilled users had to resort to in-house adaption of commercial (nano)LC-MS sprayers to do CE-ESI/MS
- Hewlett-Packard (Agilent Technologies) introduced Triple Tube Sprayer (co-used by Bruker)

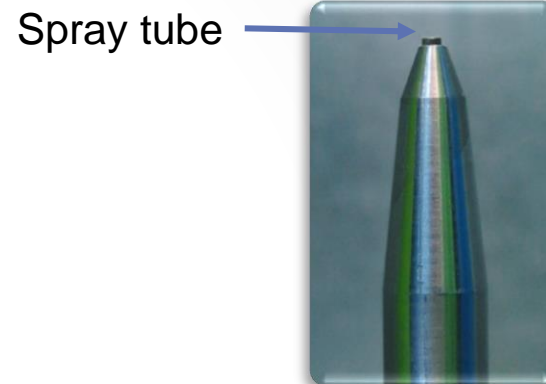
## “Triple Tube” design\*

- **Sheath solvent** is added to the CE effluent at a rate of typically 1 - 5  $\mu\text{L}/\text{min}$ . Spray becomes independent of BGE composition and EOF
- **Spray needle** (gray) is grounded. Common ground for CE and ESI. Bubbles are transported out. ES voltage provided from MS
- **Sheath solvent composition** dominates electrospray ionization chemistry
- **Compliant with different ionization modes:** ESI, APCI, APPI
- **Orthogonal configuration** (LC-MS) lets neutrals & big droplets pass



# CE-ESI/MS Coupling Retrospective

## Essentials of HP/Agilent Coaxial Sheath Solvent Flow Sprayer Concept\*



- Three tubes (CE capillary 0.36 mm o.d, spray needle 0.4 mm, i.d. 0.5 mm o.d. and nebulizer capillary, 0.8 mm i.d.) concentrically aligned and immobilized
- CE capillary continuously adjustable in axial direction
- One interface fits all MS (6xxx series)
- Fully integrated CE, ESI interface, sheath solvent delivery control and MS data acquisition and data handling software

\*EP0878021B1, Hans-Peter Zimmermann et al.



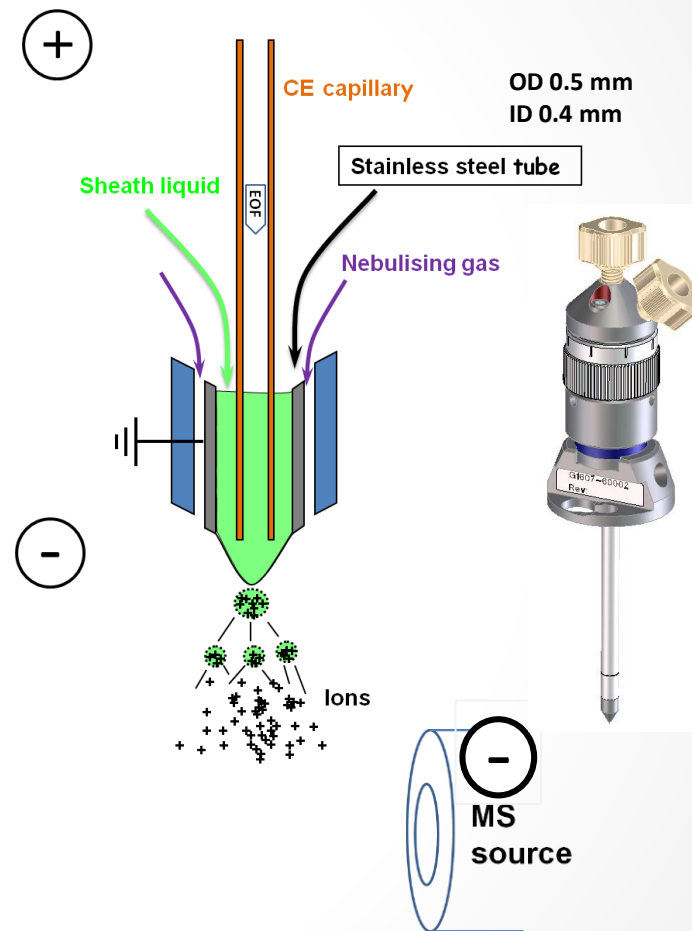
# CE-ESI/MS - Current Status

## Agilent Triple Tube Sprayer IF

- ☺ Since 1995 only complete commercial system for CE-ESI/MS
- ☺ Proven robustness and reliability
- ☺ Typical sensitivity 0.5 - 10  $\mu\text{M}$  (in sample concentration)

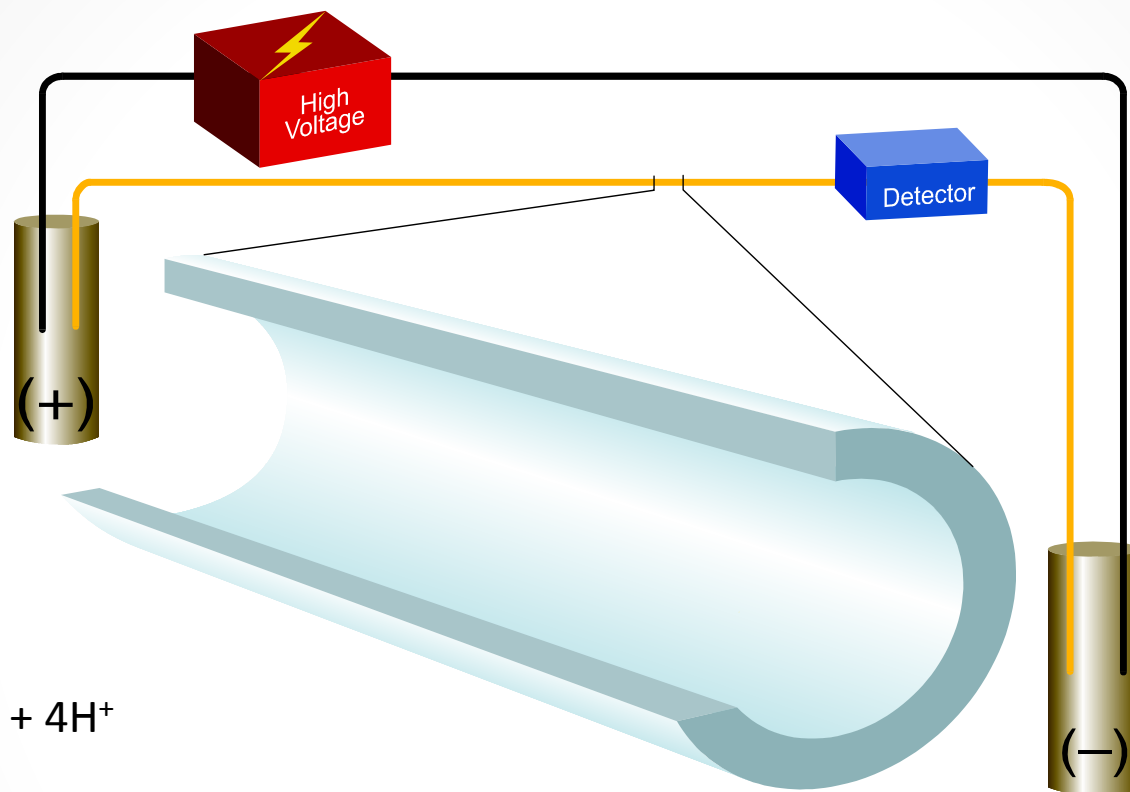
But ....

- **Sensitivity becomes compromised**
  - Concentration sensitive detection!
  - Solute concentration is reduced 5 - 50x by the sheath solvent depending on the actual EOF
  - Because of the higher flow rate no nano-electrospray (<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**



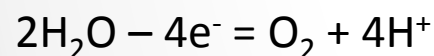
# Electrochemical Reactions in CE @ Platinum Electrodes\*

## Normal Polarity



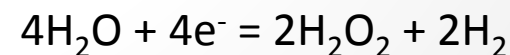
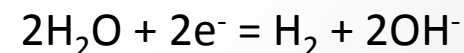
Inlet Anode

Oxidation



Outlet - Cathode

Reduction



\* Courtesy David Chen, University of British Columbia

# Electrochemical Reactions in CE-MS @ SST Electrode\*

## Reversed Polarity

CE-MS of anions

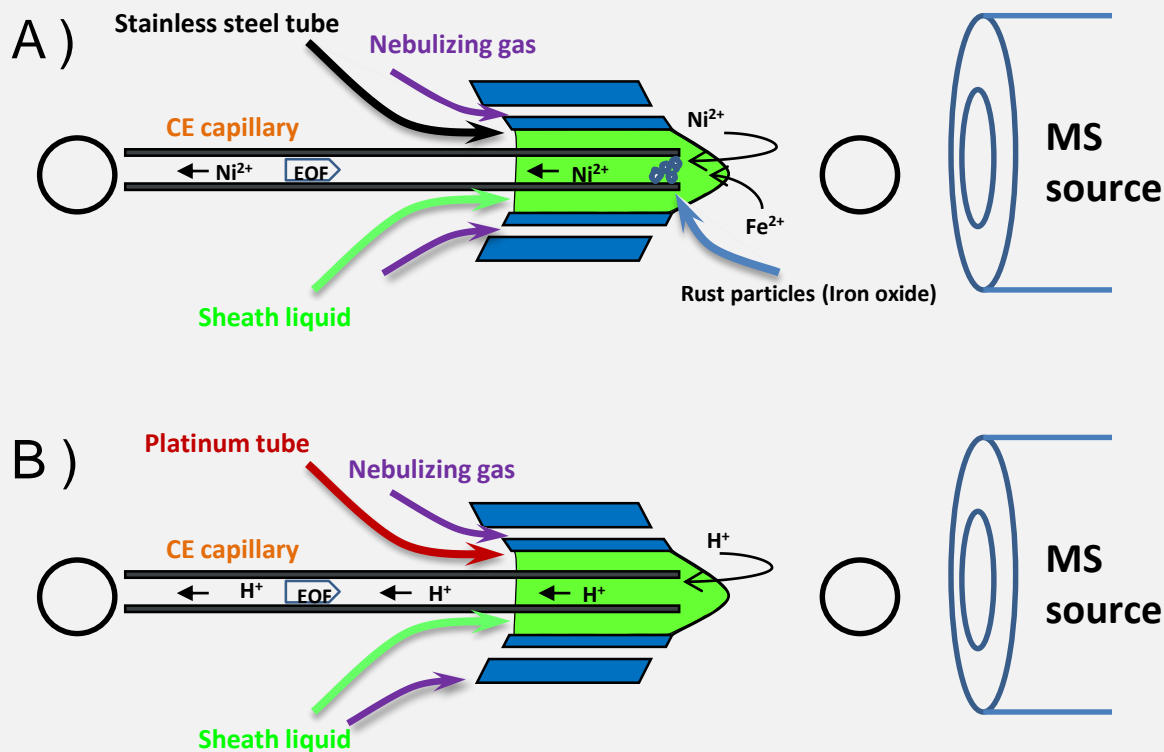
Capillary coated with a cationic layer

Reverse polarity → EOF and mobility towards the outlet

Spray needle becomes the anode

Since water is “nobler” than Fe and Ni, the metal becomes oxidized

Platinum Electrospray needle assembly for CE-MS, G7100-60041



\* Soga et al., *Anal. Chem.* 2009, 81, 6165–6174

# Current Status of CE-ESI/MS Coupling

## Agilent Triple Tube Sprayer IF

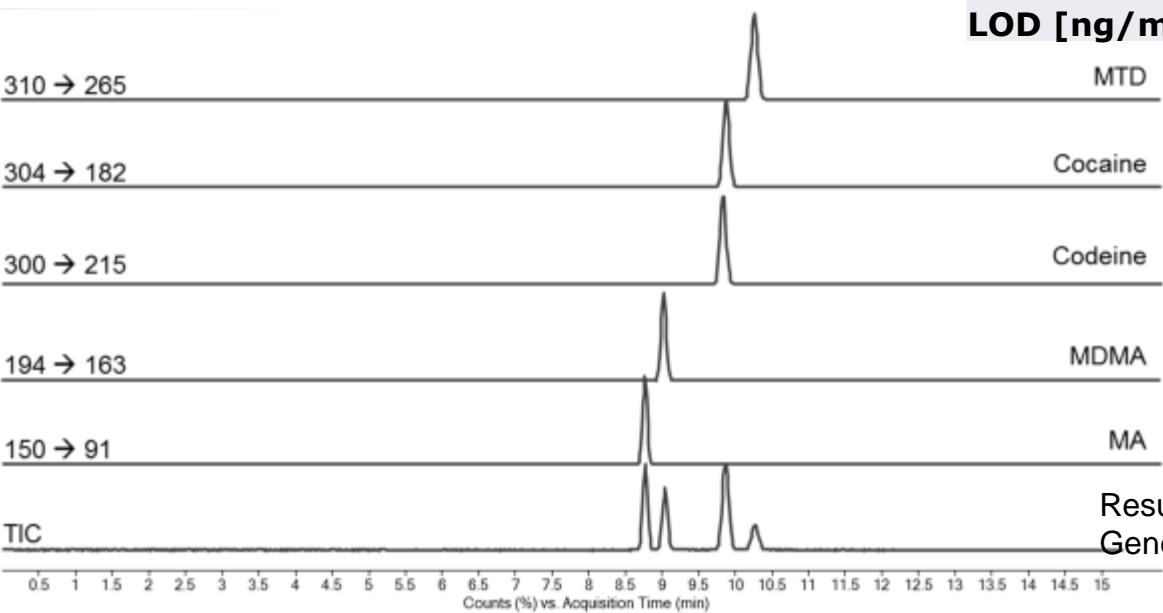
### Improvements by Agilent

- Optimized sprayer geometry/Pt needle avoiding corrosion
- Apply LC-MS Jetstream IF technology
- Higher ion capture with (Agilent 6x90 MS series)
  - Hexabore inlet capillary
  - Ion funnel



### Aqueous Standard Drugs of Abuse

	MA	MDMA	Codeine	COC	MTD
<b>LOD [ng/mL]</b>	<b>0.5</b>	<b>0.5</b>	<b>5</b>	<b>2</b>	<b>50</b>

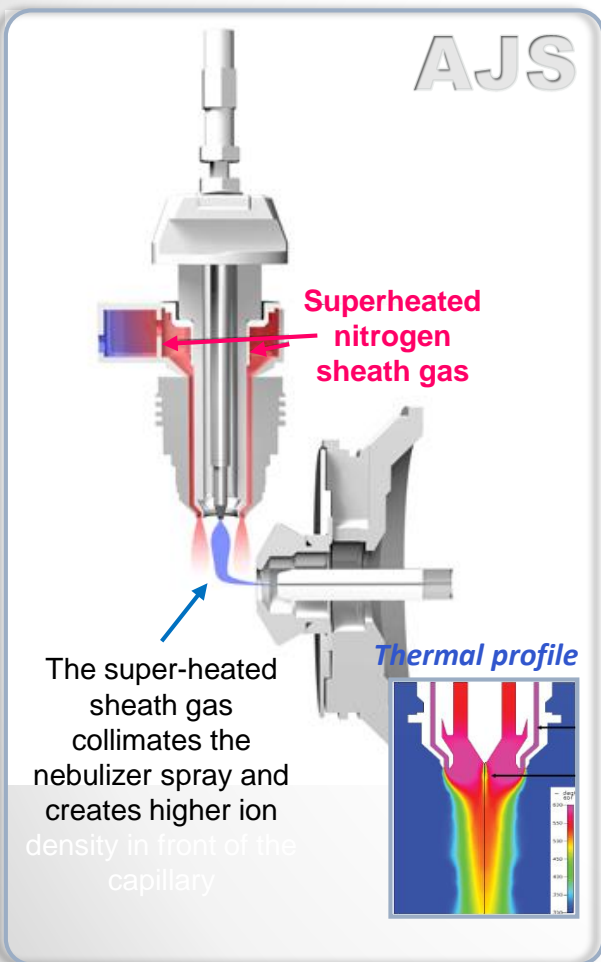


Sensitivity: ~ 2 - 200 nM concentration in sample

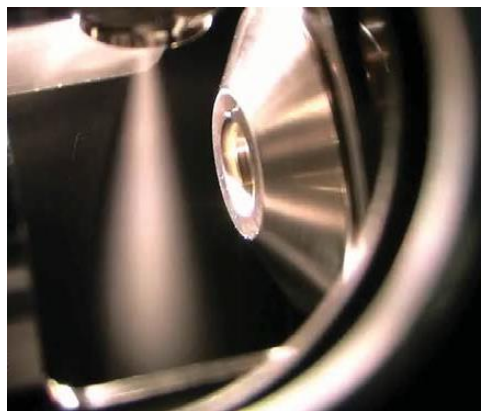
Results courtesy I. Kohler et al., University Geneva

# Agilent Jet Stream Technology

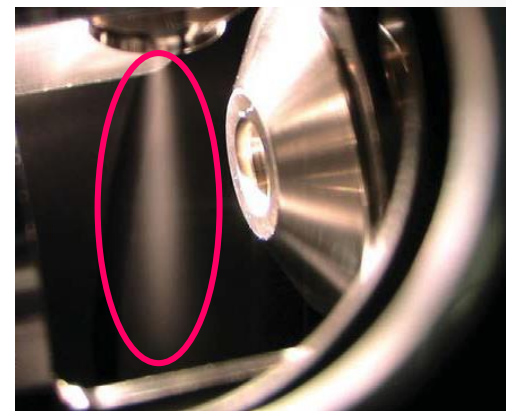
Available on Agilent 6000 Series MS Systems



25 °C



350 °C



*Agilent Jet Stream Thermal Gradient Focusing Technology, Technical Note 5990-3494EN (2009)*

LC-MS ✓

CE-MS ?

# Recent Developments in CE-MS Coupling

- Porous tip approach\*
- Micro flow-through vial\*\*
- EOF driven borosilicate glass sprayer\*\*\*
- Fused silica sprayer\*\*\*\*

\*M. Moini, Anal. Chem., **79**, 4241 (2007)

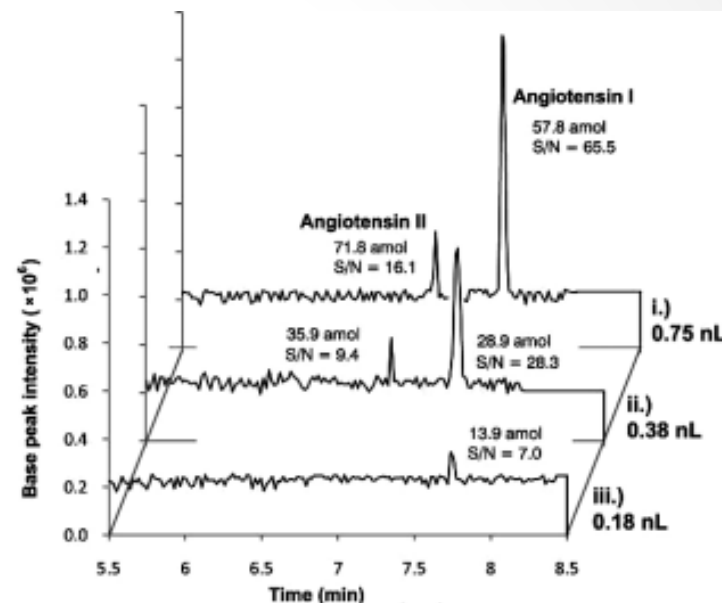
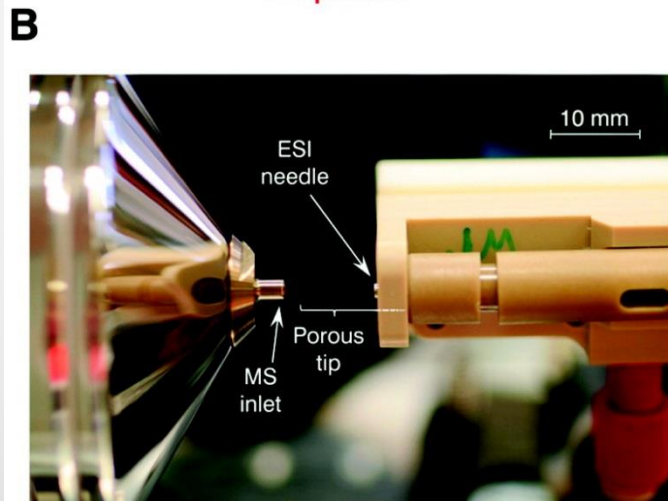
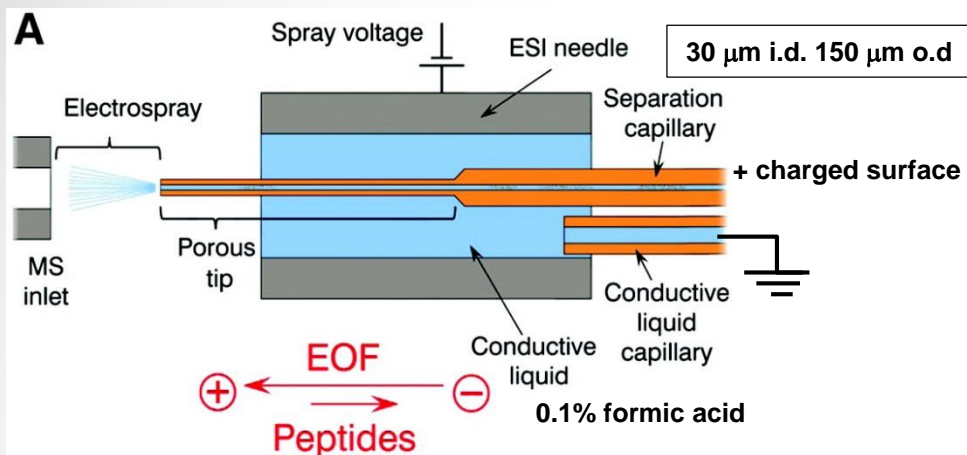
\*\*D.D.Y. Chen et al., Anal. Chem. **83**, 4916 (2011)

\*\*\*N. Dovichi et al., Rapid Comm. Mass Spec., **24**, 2554 (2010)

\*\*\*\*R.D. Smith et al., Anal. Chem., **84**, 10395 (2012)

# Recent Developments in CE-MS Coupling

## Porous Tip Approach (Moini, Univ. Texas)



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

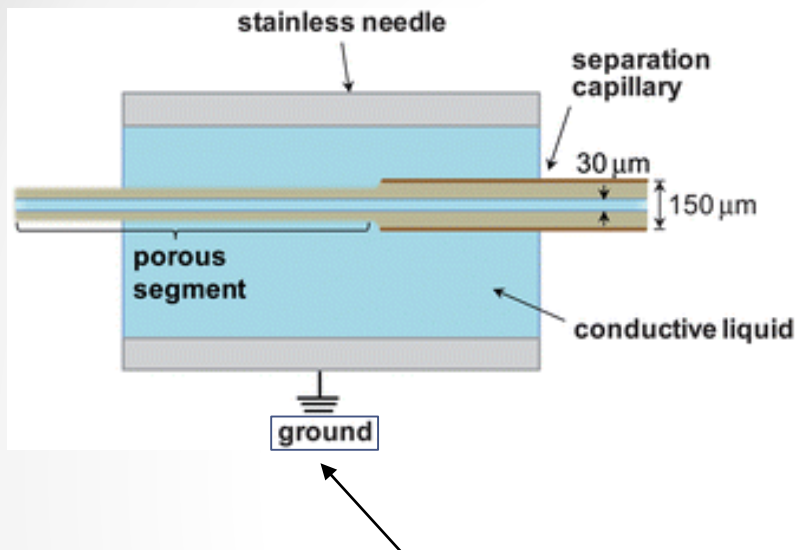
The high-sensitivity porous sprayer interface (A) schematic and (B) photograph of the prototype interface.

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



# Recent Developments in CE-MS Coupling

## Comparison Coaxial Sheath Flow and Porous Tip (T. Soga et al.)



VS.

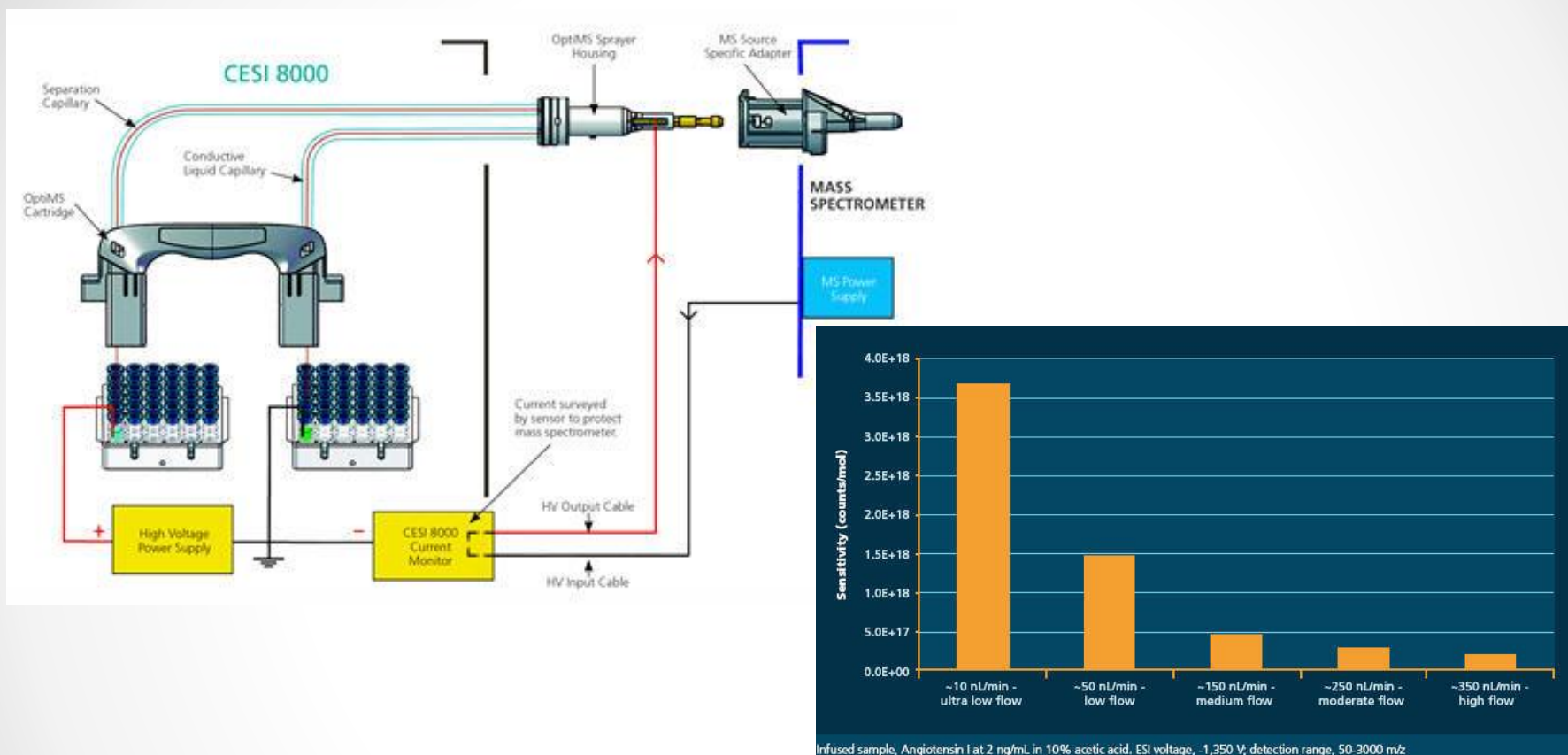


<b>System:</b>	Agilent 6220 Accurate-Mass TOF LC/MS with Beckman Coulter PA800 plus CE and Agilent G7100 series CE respectively
<b>Sample:</b>	Cationic metabolites, 2.3 and 2.6 nL injected resp.
<b>BGE &amp; Contact:</b>	1 M formic acid
<b>BGE &amp; Sheath:</b>	1 M formic acid, MeOH/Water with 0.1% hexakis
<b>Capillaries</b>	50 μm i.d. and 30 μm i.d. resp.
<b>Sensitivity:</b>	10-100 nM concentration in sample with porous tip
<b>Relative:</b>	<u>0.2 – 20x</u> sheathless/coaxial sheath flow
<b>Robustness:</b>	180 successive runs

T. Soga et al., Analyst, **137**, 5026 (2012)



# Sciex Separations\* CESI 8000 ESI Module



Capillary's distal end is porous to allow ion flow

Electrical contact for the CE is achieved through an ESI needle filled with conductive fluid

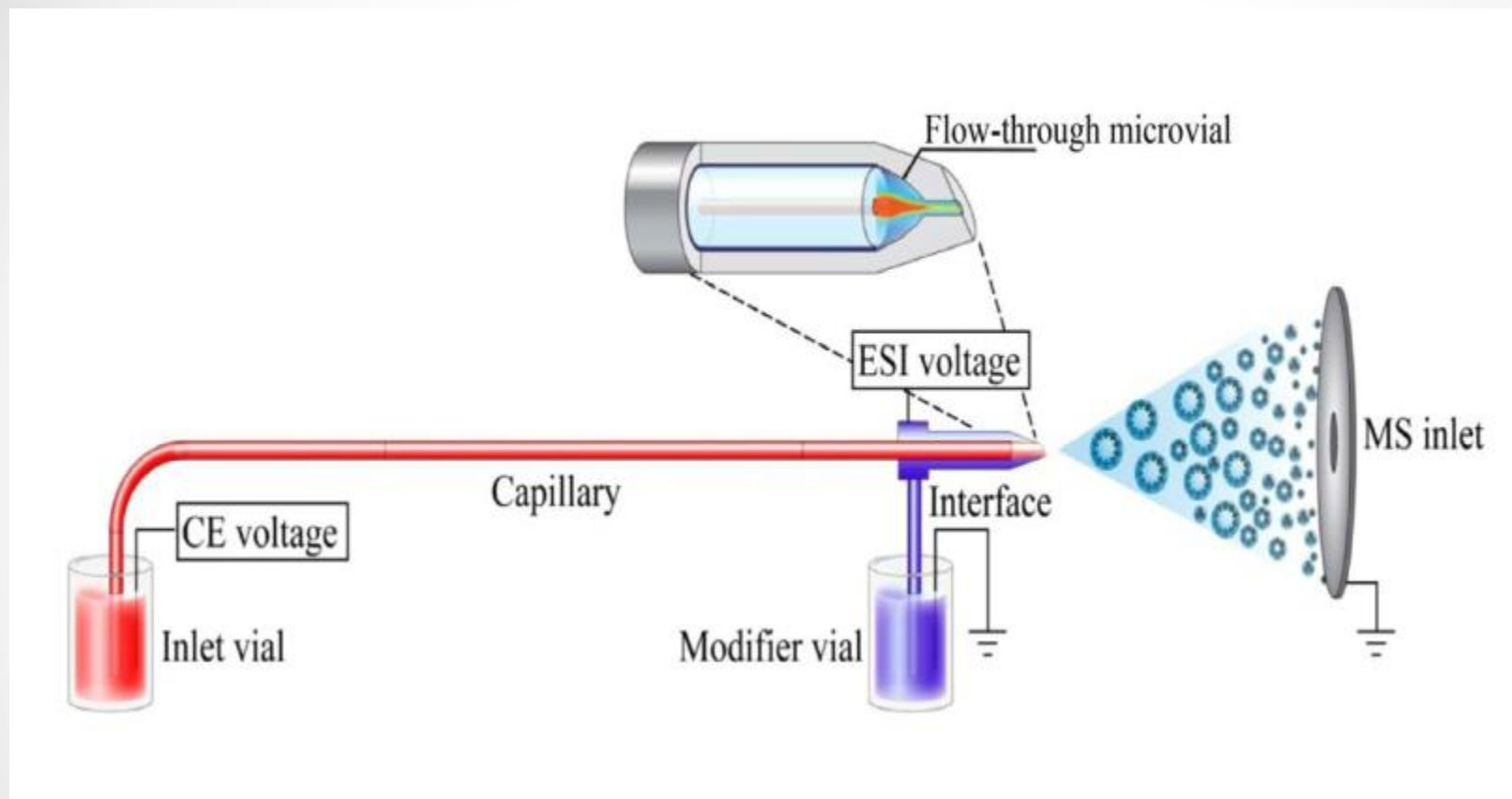
ESI's electrical contact is achieved through the protruding capillary tip

Low flow at the tip terminus instantly generates a fine spray when ESI voltage is applied

\*Formerly Beckman-Coulter

# Recent Developments in CE-MS Coupling

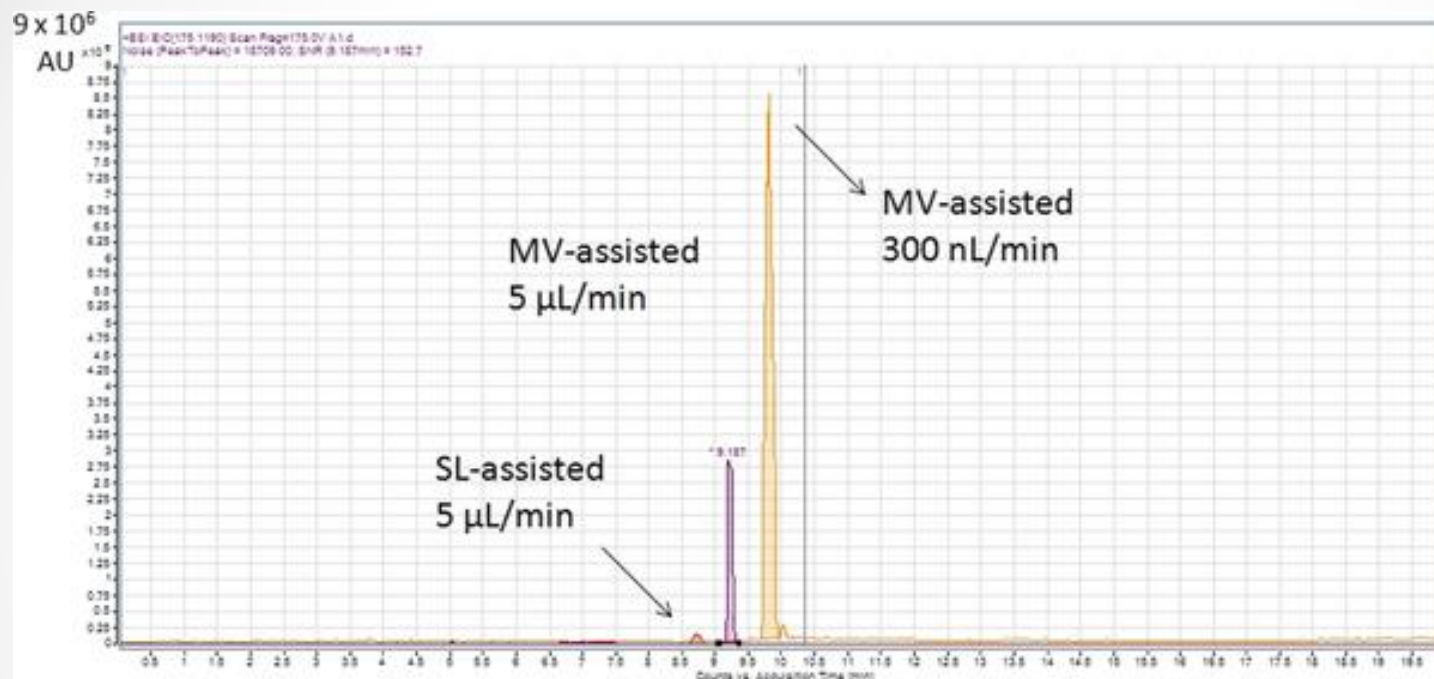
Micro Flow-Through Vial (D.D.Y. Chen et al.)



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

# Recent Developments in CE-MS Coupling

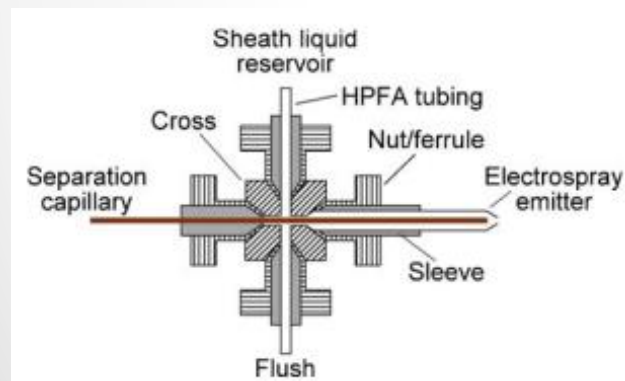
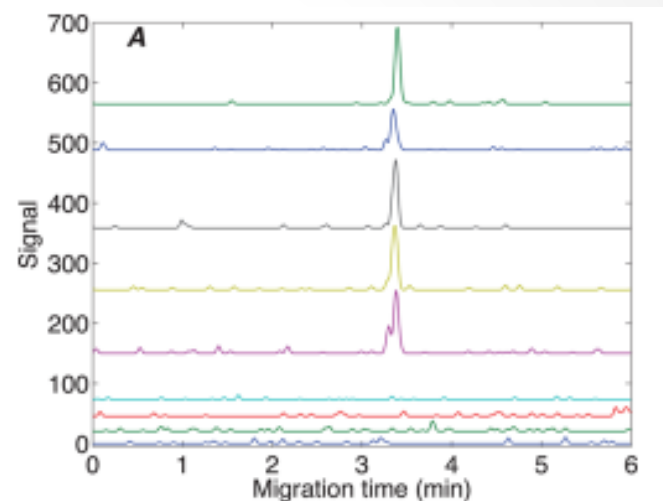
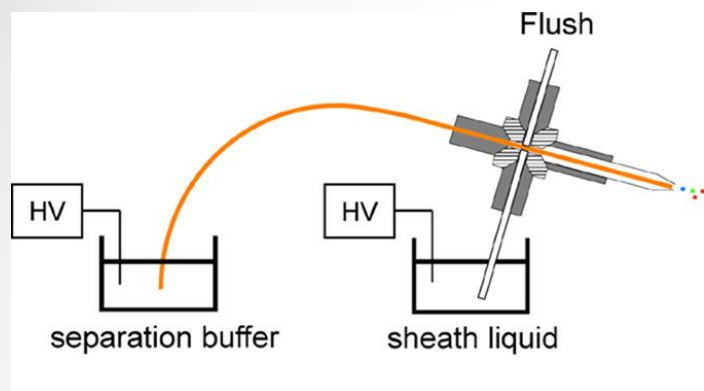
## Micro Flow-Through Vial (D.D.Y. Chen et al.)



Comparison of conventional CE-MS interface with the MV-assisted interface operated at different SL flow rates. CE conditions are as follows: capillary length 1 m, capillary diameter 50  $\mu$ m, injection 30 s, 35 mbar, BGE 10 % acetic acid, condition, separation voltage 30 kV. Detection was carried out with Agilent Technologies 6550 iFunnel-Q-TOF-MS. Analyte m10 M arginine.

# Recent Developments in CE-MS Coupling

## EOF Driven Sprayer (N. Dovichi et al)



FS separation capillary 50x150  $\mu\text{m}$   
Borosilicate emitter capillary 0.75x1 mm, orifice 5  $\mu\text{m}$   
 BGE 10 mM ammonium acetate, pH 5.5  
 Sheath solvent MeOH/0.1% formic acid  
 Sample: short peptides

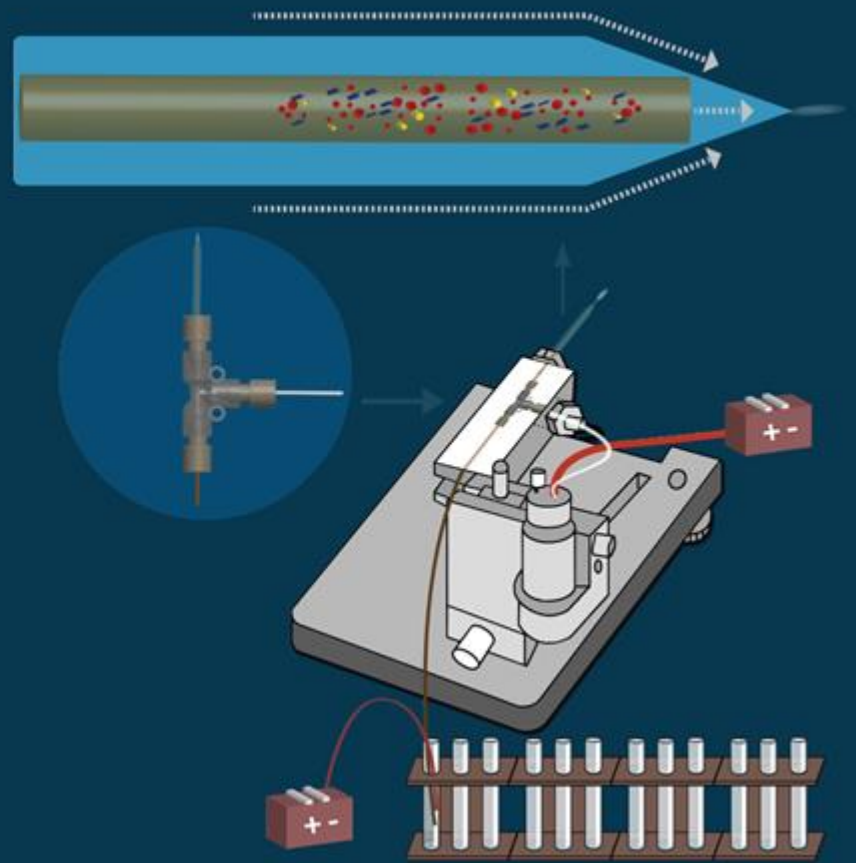
Sensitivity: < 1 nM in sample concentration

# Recent Developments in CE-MS Coupling

## CMP Scientific – EMASS-II Ion Source\*

CMP Scientific's EMASS-II ion source incorporates an EOF driven sheath liquid electrospray emitter technology. In this interface, the separation capillary terminus makes contact with the electrospray emitter inside, forming a small volume which acts as the capillary electrophoresis outlet vial. Sheath liquid solution is introduced through a tee junction at a flow driven by borosilicate glass surface EOF, thus minimizing dilution of the CE effluent in order to maximize sensitivity. Compared with a typical sheath-flow CE-MS interface, this innovative design results in 50-100 fold or higher increase in mass spectrometry signal.

- ◇ EOF-driven sheath liquid flow
- ◇ Nanoflow sensitivity
- ◇ High electrospray efficiency
- ◇ Extremely robust

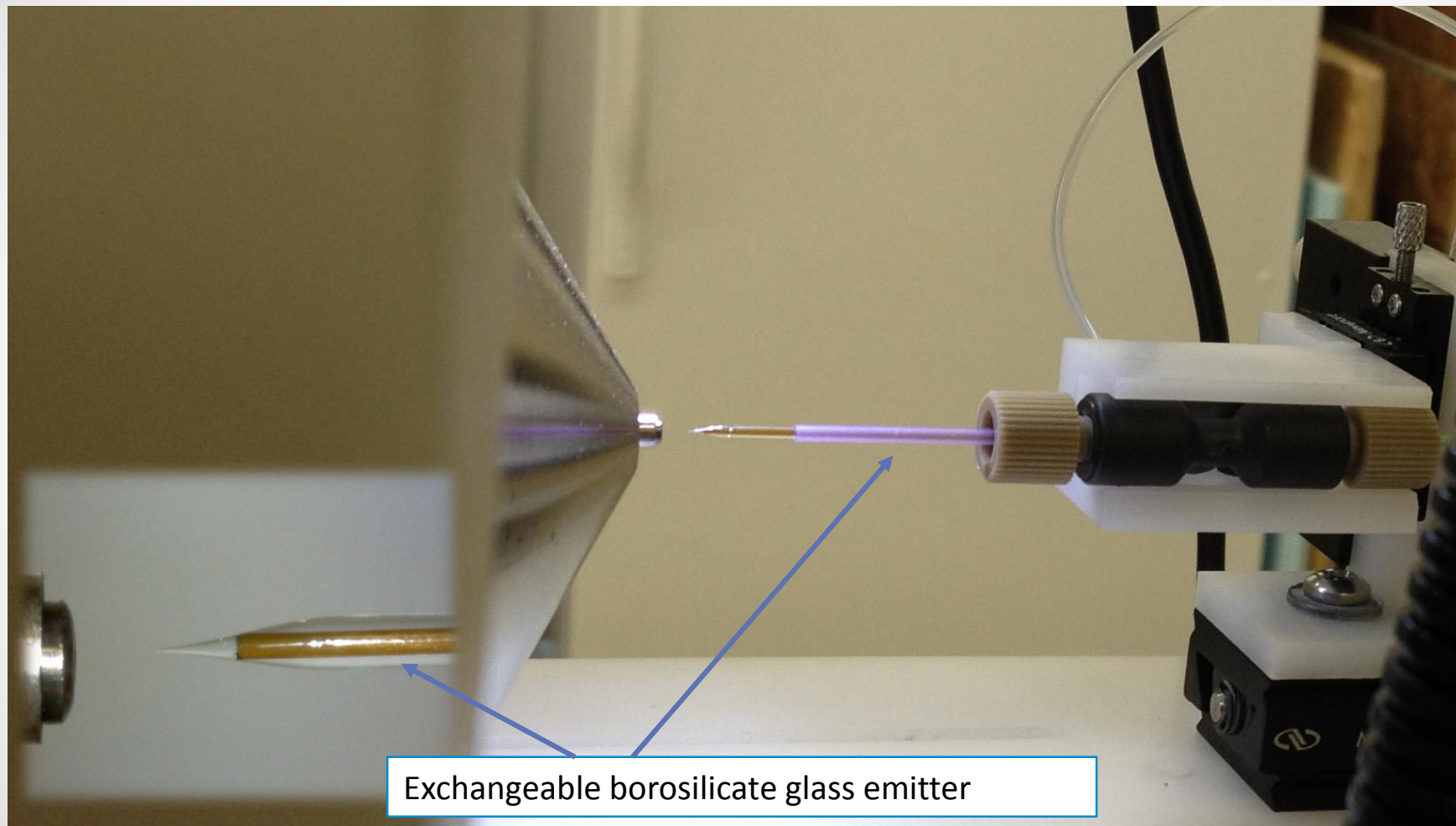


\*Slide provided by James Xia, CMP Scientific



# Recent Developments in CE-MS Coupling

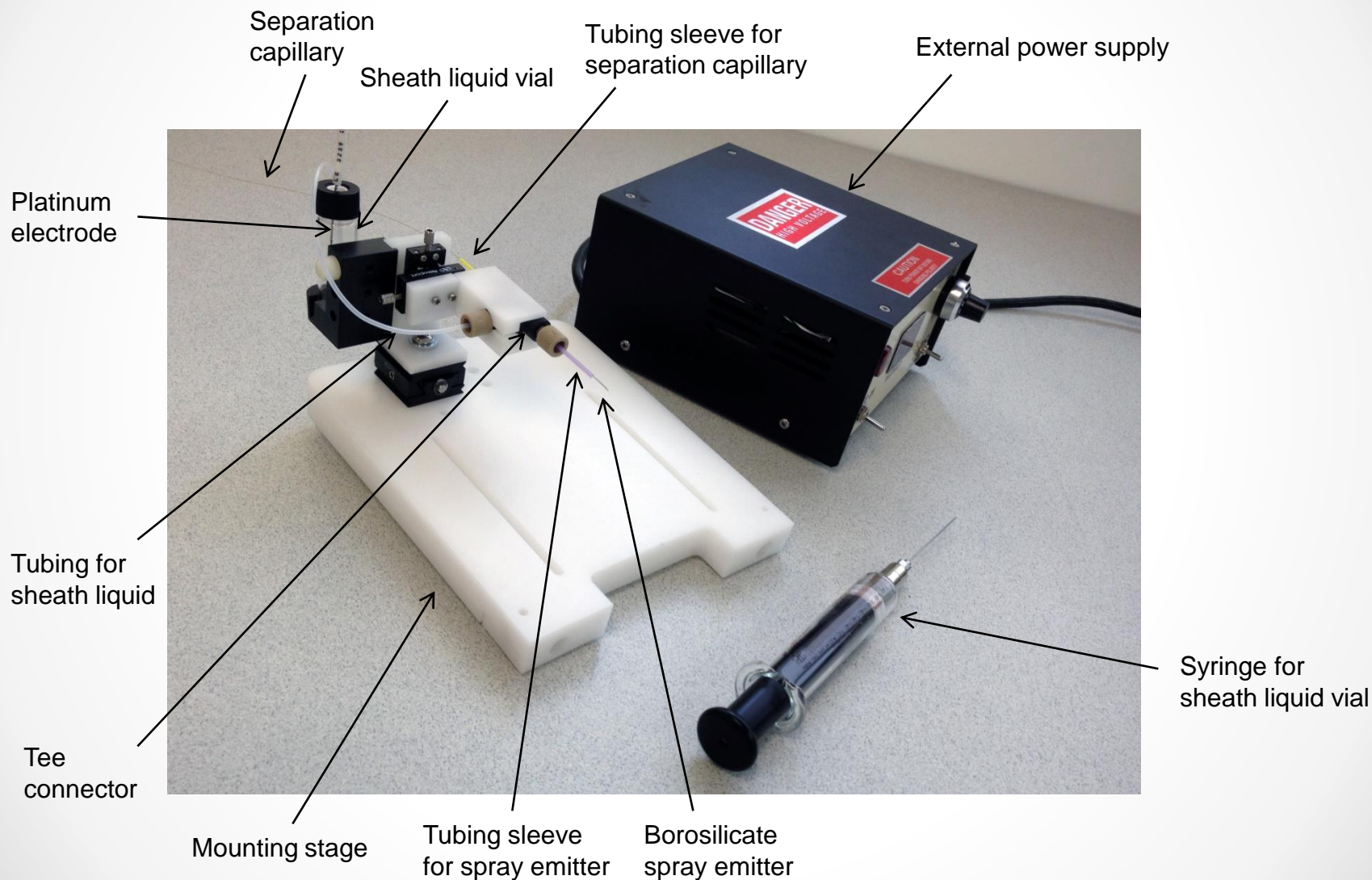
**CMP Scientific – EMASS-II Ion Source\***



**\*Slide provided by James Xia, CMP Scientific**

# Recent Developments in CE-MS Coupling

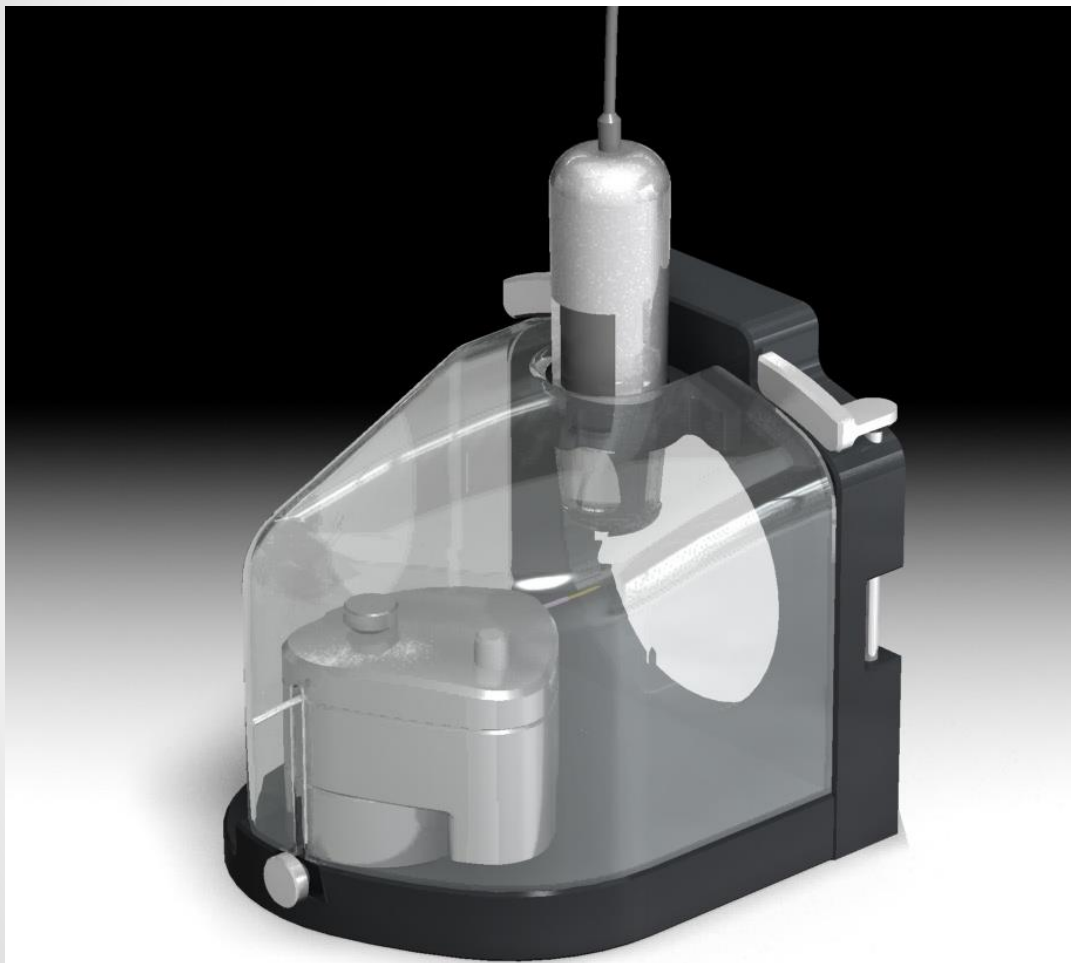
## CMP Scientific – EMASS-II Ion Source\*



\*Slide provided by James Xia, CMP Scientific

# Recent Developments in CE-MS Coupling

**CMP Scientific – EMASS-II Ion Source\***

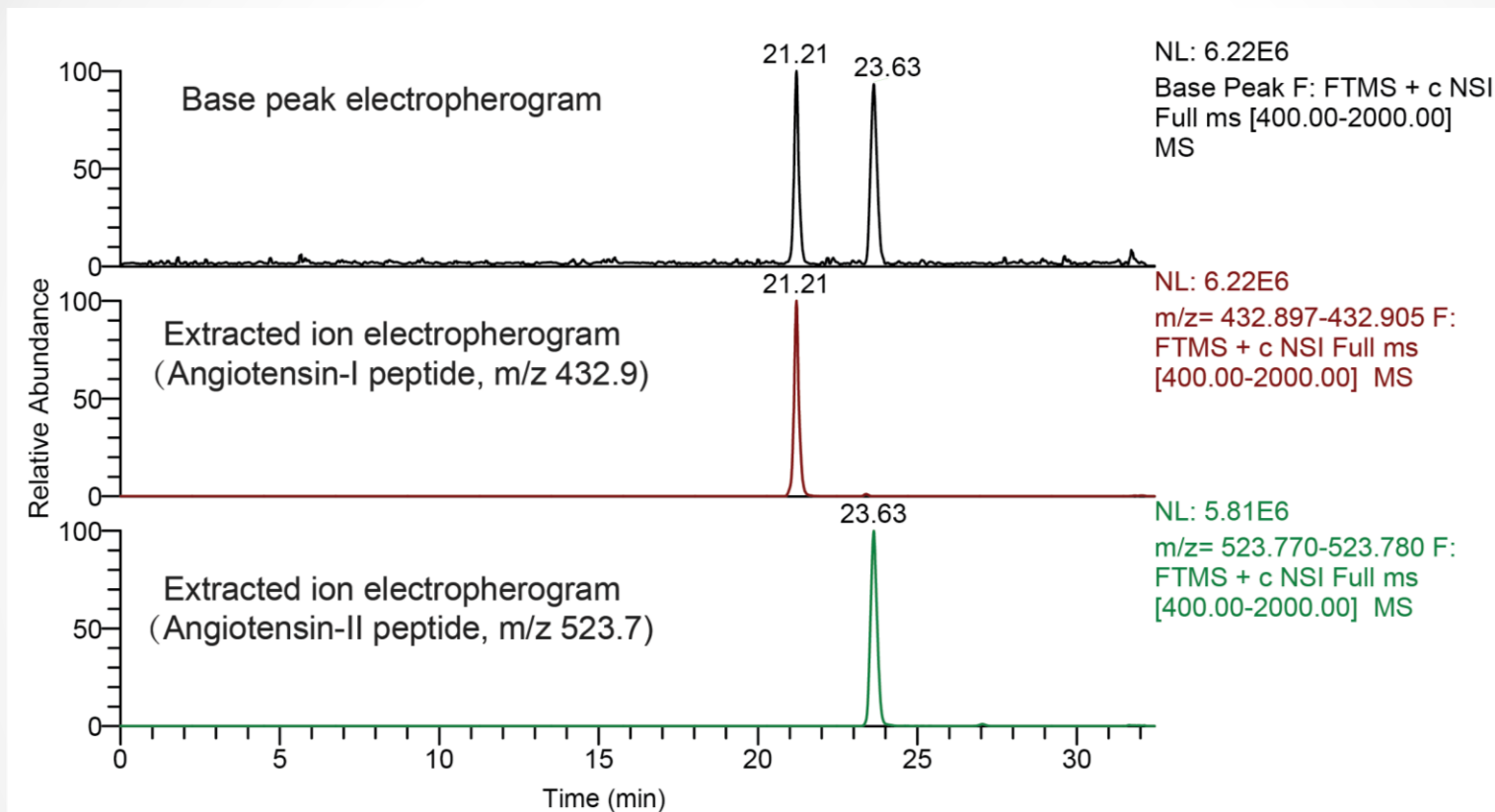


\*Slide provided by James Xia, CMP Scientific



# Recent Developments in CE-MS Coupling

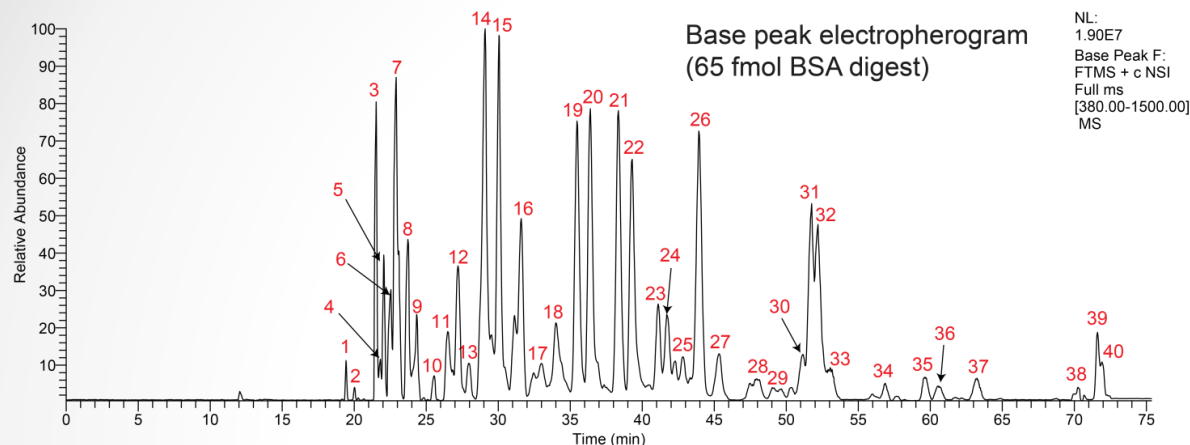
## CMP Scientific – EMASS-II Ion Source



CZE-ESI-MS analysis of Angiotensin I & II peptide mixture. Sample : Angiotensin I & II, 1 µg/mL of each in 0.05% formic acid, 1% methanol. CE: Beckman P/ACE MDQ, MS: Thermo LTQ-Orbitrap xL, CE-ESI-MS ion source: CMP Scientific EMASS-II Ion Source. Separation capillary: 150 µm OD x 30 µm ID x 90 cm, LPA-coated. Spray emitter: borosilicate glass, 1 mm OD, 0.75 mm ID, 15 µm tip. BGE: 30% acetic acid, 2 mM TETA. Sheath liquid: 0.5% formic acid, 10% methanol. Sample injection: 30 nL (20 psi, 10 s). Separation voltage: 30 KV. Electrospray voltage: 1.7 KV. Distance from emitter tip to mass spec: 2.0 mm.

# Recent Developments in CE-MS Coupling

## CMP Scientific – EMASS-II Ion Source



BSA tryptic digest  
1  $\mu$ M

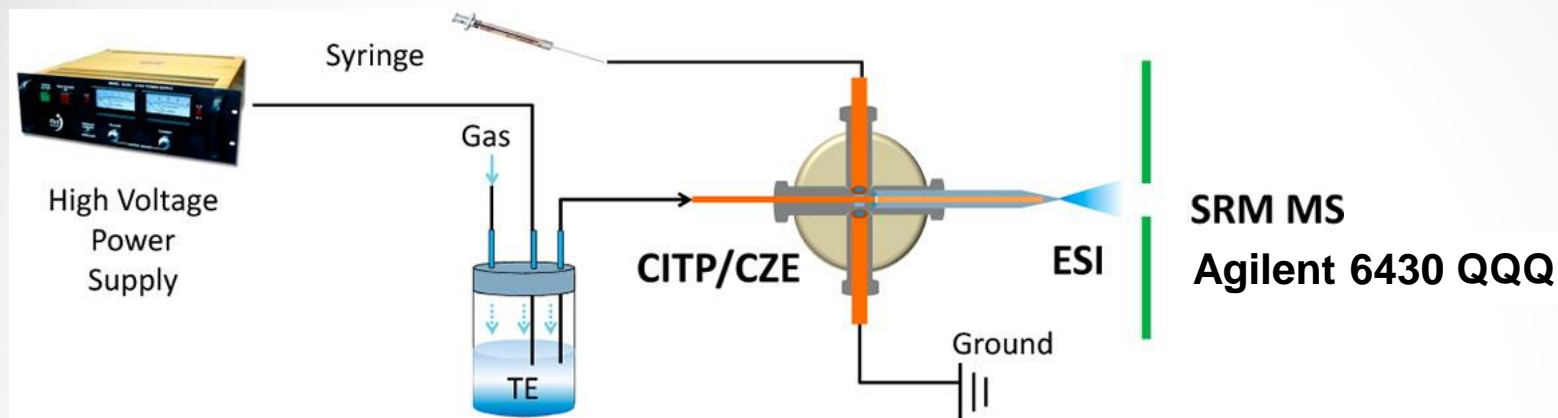
Sequence Coverage = 88%

DTHKSEIAHR FKDLGEEHFK GLVLIAFSQY LQQCPFDEHV KLVNELTEFA KTCVADESHA<sup>60</sup>  
 27 3 26 23 26 16  
GCEKSLHTLF GDELCKVASL RETYGDMADC CEKQEPERNE CFLSHKDDSP DLPKCLKDPN<sup>120</sup>  
 15 13 33 11 13  
TLCDEFKADE KKFWGKLYE IARRHPYFYA PELLYYANKY NGVFQECQA EDKGACLLPK<sup>180</sup>  
 17 1 21 19 17  
IETMREKVL SSARQRLRCA SIQKFGERAL KAWSVARLSQ KFPKAEFVEV TKLVTDLT<sup>240</sup>  
 16 5 22 20  
HKECCHGDL ECADDRADLA KYICDNQDTI SSKLKECCDK PLLEKSHCIA EVEKDAIPEN<sup>300</sup>  
 6 32 8 10  
LPPLTADFAE DKDVCKNYQE AKDAFLGSFL YEYSRRHPEY AVSVLLRLAK EYEATLEECC<sup>360</sup>  
 18 17 35 7  
AKDDPHACYS TVFDKLKHLV DEPQNLIKQN CDQFEKLGEY GFQNALIVRY TRKVPQVSTP<sup>420</sup>  
 27 9 14 22 31 19  
TLVEVSRSLG KVGTRCCTKP ESERPCTED YLSLILNRLC VLHEKTPVSE KVTKCCTESL<sup>480</sup>  
 25 34 4 24  
VNRRPCFSAL TPDETYVPKA FDEKLFTFHA DICTLPDTEK QIKKQTALVE LLKHKPKATE<sup>540</sup>  
 20 21 12 23  
EQLKTMENF VAFVDKCCAA DDKEACFAVE GPKLVVSTQT ALA<sup>583</sup>  
 30 21 39

CZE-ESI-MS analysis of BSA tryptic digest. Sample : 0.35 mg/mL BSA digest in 30% acetonitrile and 0.04% formic acid. CE: PrinCE 560, MS: Thermo LTQ-Orbitrap Velos, CE-ESI-MS ion source: CMP Scientific EMASS-II Ion Source. Separation capillary: 150  $\mu$ m OD x 50  $\mu$ m ID x 90 cm, LPA-coated, end etched to 60  $\mu$ m. Spray emitter: borosilicate glass, 1 mm OD, 0.75 mm ID, 25  $\mu$ m tip. BGE: 5% acetic acid. Sheath liquid: 0.5% formic acid, 10% methanol. Sample injection: 12 nL (3.6 psi, 8 s). Separation voltage: 25.5 KV. Electrospray voltage: 1.7 KV. Distance from emitter tip to mass spec: 2.0 mm.

# Recent Developments in CE-MS Coupling

## Fused Silica Sprayer (R.D. Smith et al.)



**Separation capillary:** FS 75x150  $\mu\text{m}$ , neutral coating

**Emitter capillary:** FS 200x350  $\mu\text{m}$ , end etched with HF and orifice 50  $\mu\text{m}$

**BGE:** 25 mM ammonium acetate, pH 4

**Sheath solvent and TE:** 9/1 0.1 M acetic acid/methanol

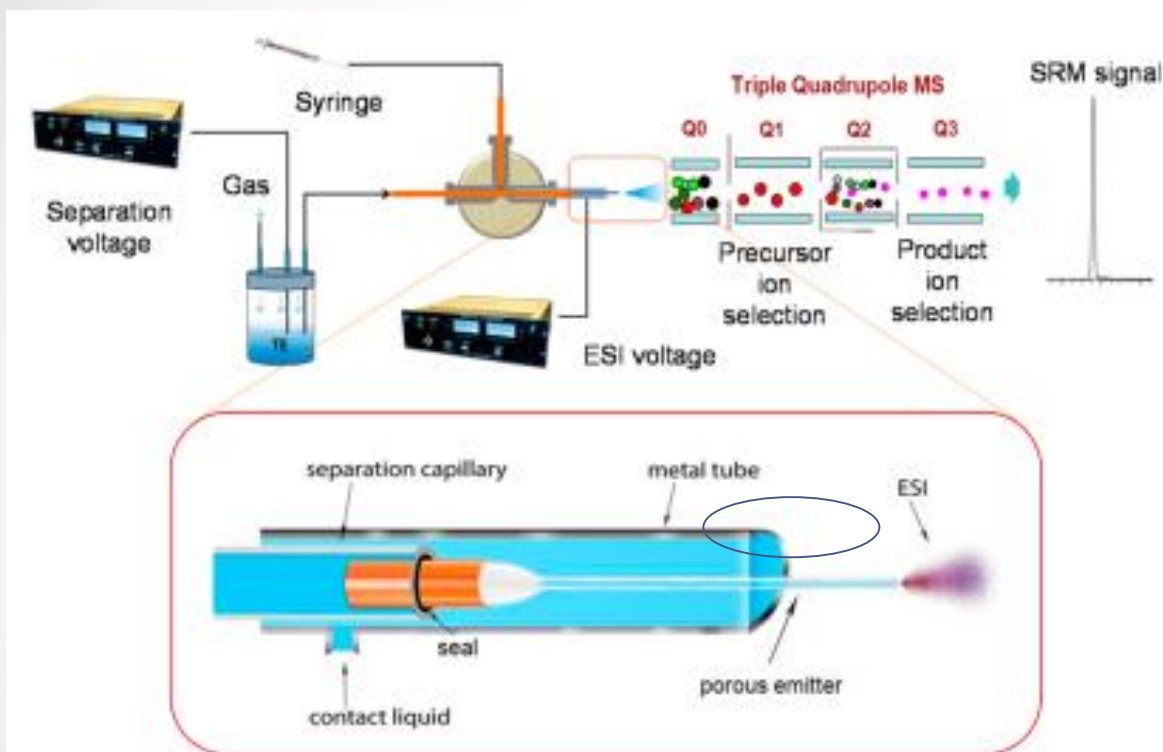
**Sample:** short peptides in BSA digest

**Sensitivity:** 10 nM 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

# Recent Developments in CE-MS Coupling

## Fused Silica Sprayer (R.D. Smith et al.)



**Separation capillary:** FS 100x360  $\mu\text{m}$ , neutral coating  
**Emitter capillary:** FS 20x95  $\mu\text{m}$ , end etched with HF and orifice 50  $\mu\text{m}$   
**ESI Voltage:** 1.7 kV applied to the metal tube  
**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, up to 3.7  $\mu\text{M}$   
**Sensitivity:** 10 nM with CIP sample pre concentration

R.D. Smith et al., Anal. Chem. 2013, 85, 7308–7315

# Assessment CE-MS Coupling

	Triple Tube Approach	Moini Approach	Chen Approach	Dovichi Approach	Smith Approach
Sensitivity (LOD)	0.5 $\mu\text{M}^{\text{b}}$ /20 nM <sup>a</sup>	20 nM <sup>b</sup>	0.2 – 5 $\mu\text{M}^{\text{c}}$	1 nM	50 nM <sup>d</sup>
Robustness/Reliability	xxx	xx	x <sup>c</sup>	x	?
Ease of Use	xxx	xx	xx	x	?
Standard Capillaries?	YES	NO <sup>e</sup>	YES	NO <sup>e</sup>	NO <sup>e</sup>
Commercially Available?	Agilent Technologies	Sciex Separations	NO	CMP Scientific	NO

a. achievable with best MS equipment

b. See table 1 in, R. Ramautar et al., Anal. Chem., **84**, 885 (2012) and T. Soga et al., Analyst, **137**, 5026 (2012)

c. improvements needed

d. In combination with CIP

e. Special, expensive, capillaries (I.D., emitter tip), wall coating for reliable EOF needed

# Future of CE-MS?

- Obtaining highest sensitivity remains top objective; but...
  - Unlike HPLC, CE has limited sample volume loading capacity.
  - In contrast to SPE, using sweeping or cITP methods is regarded “difficult”.
  - Given the same amount entered into the separation device, CE-MS will give higher response than in LC-MS!
  - The premier user’s interest though is the analyte concentration in the sample
  - Therefore, CE-MS will be the preferred choice for measurement of polar/charged analytes in very small sample volume
- Conventional coaxial sheath solvent flow IF pairs adequate sensitivity (with up-to-date MS) with ease of use and robustness
- Porous tip and EMASS-II IF seem promising (but expensive) pathways towards higher sensitivity CE-ESI/MS.
- Commercialization (affordable) will be the key for success of new sheathless CE-ESI/MS coupling methods

# Further reading: CE Primer

For more detailed information on CE and CE-MS please request the free Agilent Technologies Primers on:

- High performance Capillary Electrophoresis  
Pub. Number: 5990-3777EN
- Capillary Iso-Electric Focusing  
Pub. Number: 5991-1660EN

# Acknowledgements

- Paul Goodley for providing insights in the development of the triple tube IF
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- David Chen, University of British Columbia, Canada
- James Xia, CMP Scientific, Hoboken, USA