A BRIEF REVIEW OF CE-MS ELECTROSPRAY INTERFACING: RETROSPECTIVE, CURRENT STATUS AND NEW DEVELOPMENTS

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Outline

- A brief retrospective of coaxial sheath solvent CE-ESI/MS coupling.
- Current status of CE-ESI/MS coupling by coaxial sheath flow interface.
- New developments in CE-ESI/MS coupling; promises and reality.
- Future of CE-MS?

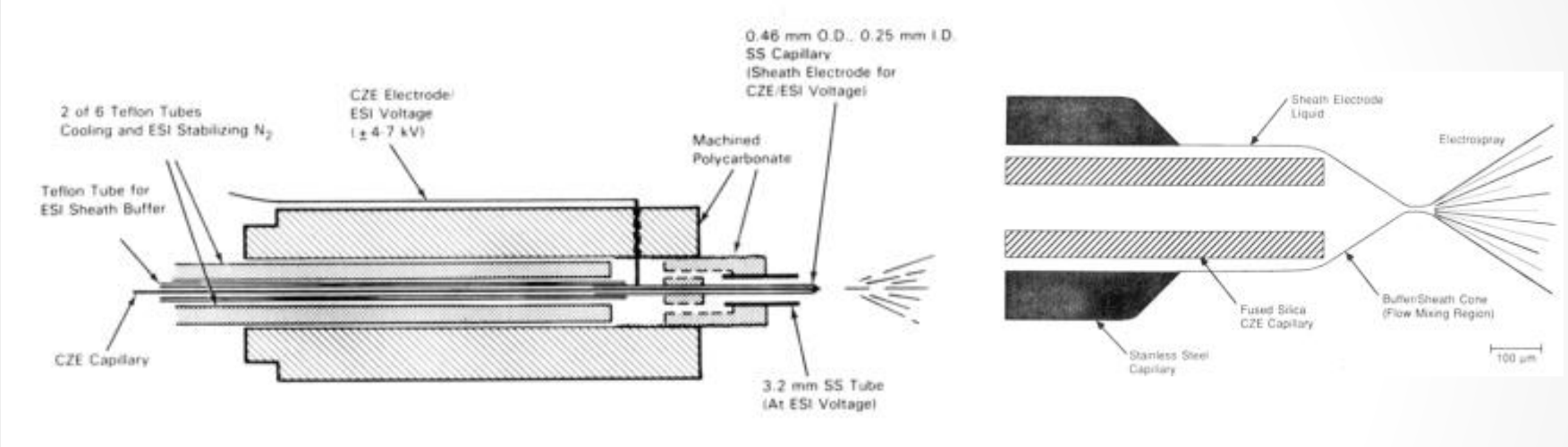
CE-ESI/MS Coupling Retrospective

Main challenges specific for CE-ESI/MS to surmount:

- No outlet vial/end electrode available when spraying into an MS
- How to apply the electrospray field between CE capillary exit and MS inlet?
- In CE, currents are typically 100-1000x larger than electrospray current; how to drain this current
- In contrast to LC-ESI/MS, the solvent flow in CE (EOF) depends on its composition. Will impart the optimization of CE separation
- Incompatibility of BGE's with non-volatile constituents and vacuum detection. Eventually the BGE used, will be suboptimal for CE separation

CE-ESI/MS Coupling Retrospective

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

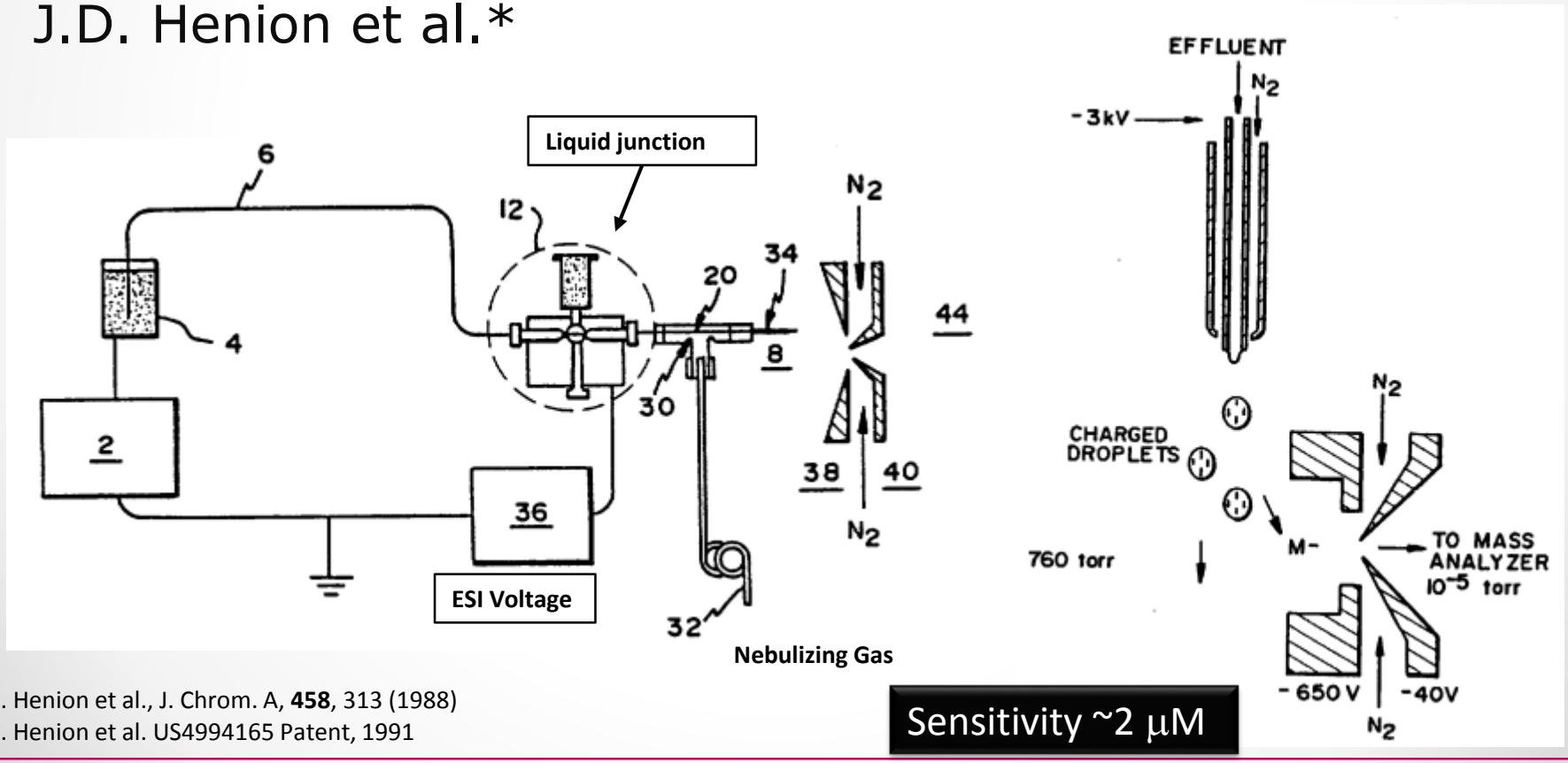


True Electrospray
Liquid electrical contact
Delivery of a sheath solvent to establish stable spray

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 Retrospective

- 1988; Initial work with coaxial sheath solvent, R.D. Smith et al.
- 1988; Ion spray approach with liquid junction, J.D. Henion et al.*

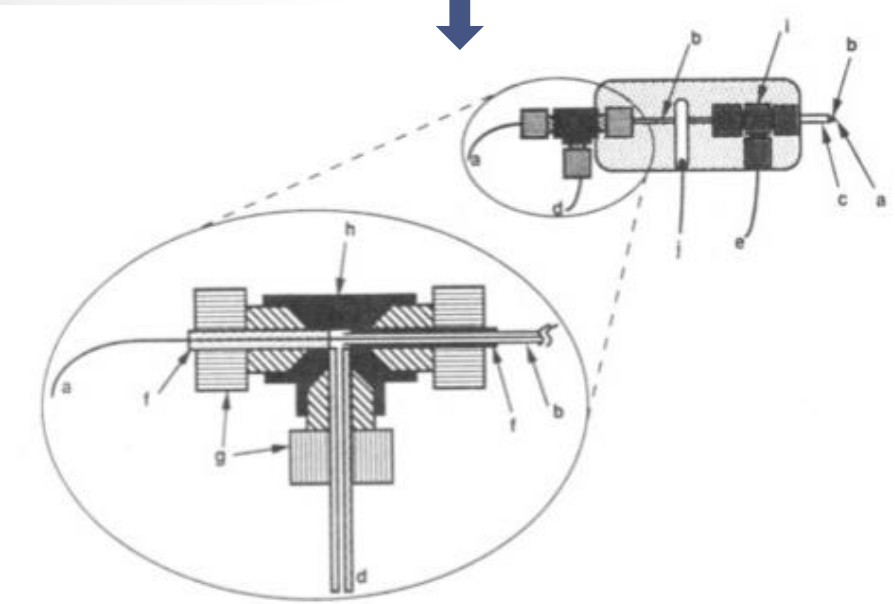


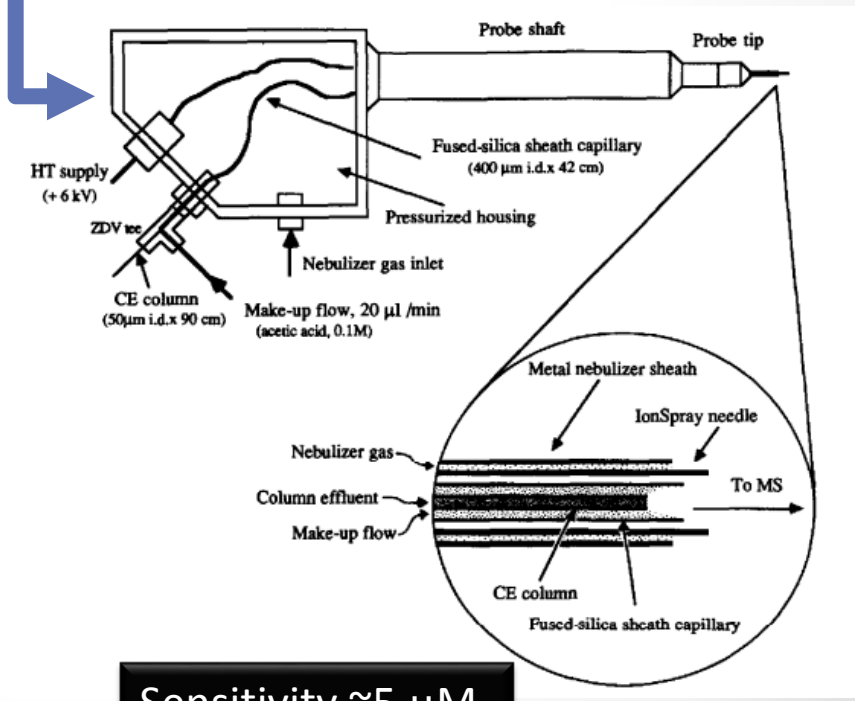
Sensitivity ~2 μM

J.D. Henion et al., J. Chrom. A, **458**, 313 (1988)
J.D. Henion et al. US4994165 Patent, 1991

CE-ESI/MS Coupling Retrospective

- 1988; Initial work with coaxial sheath solvent, R.D. Smith et al.
- 1988; Ion spray approach with liquid junction, J.D. Henion et al.
- 1991; Combination of coaxial sheath solvent and ion spray interface for CE-ESI-MS, P. Thibault et al.* and 1994,Henion et al.**





Sensitivity ~5 µM

*P. Thibault et al., J. Chrom., **542**, 483 (1991)
** J.D. Henion et al., Anal. Chem. **66**, 2103 (1994)

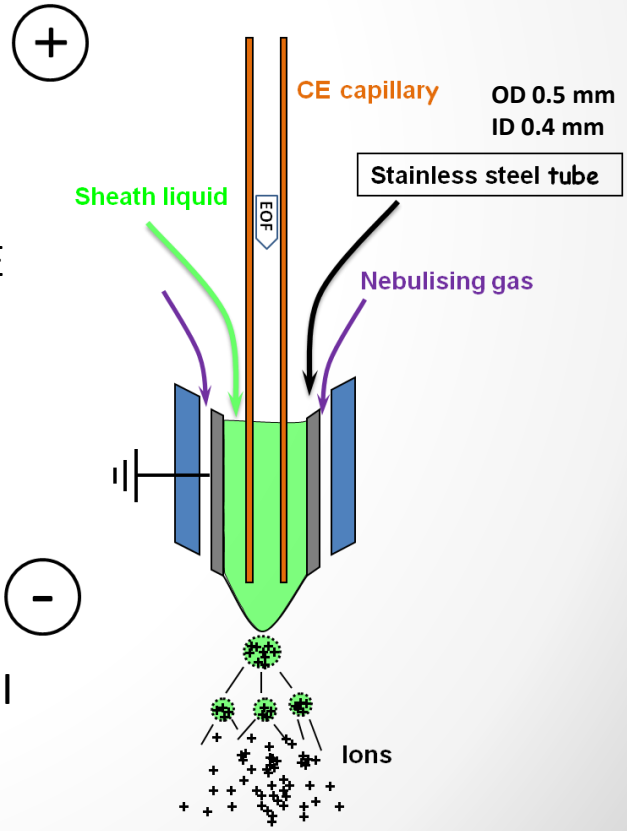
CE-ESI/MS Coupling Retrospective

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 µL/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 Spayer Concept*

- Three tubes (CE capillary 0.36 mm o.d, spray needle 0.4 mm i.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 Coupling Retrospective

Agilent 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 is compromised

➔ Concentration sensitive detection!

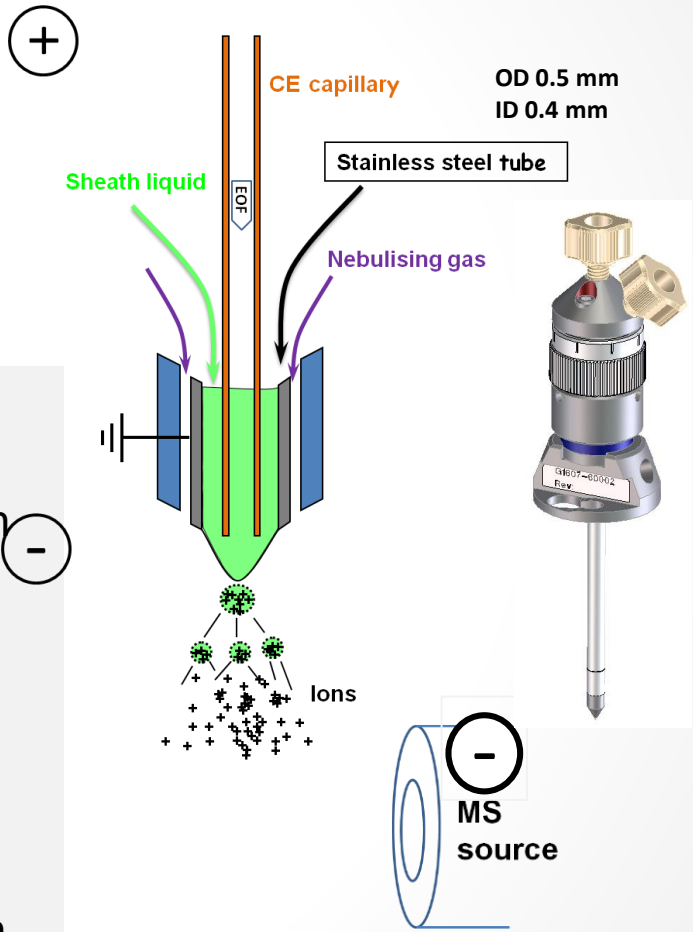
➔ Solute zone is diluted 5 - 50x with the sheath solvent depending on the EOF present

➔ With higher flow rate no nano-electrospray and ion capture reduces

Pneumatic assistance required to establish the spray

➔ Undesirable hydraulic flow is observed, which need countermeasures

Galvanic reactions on the sprayer needle



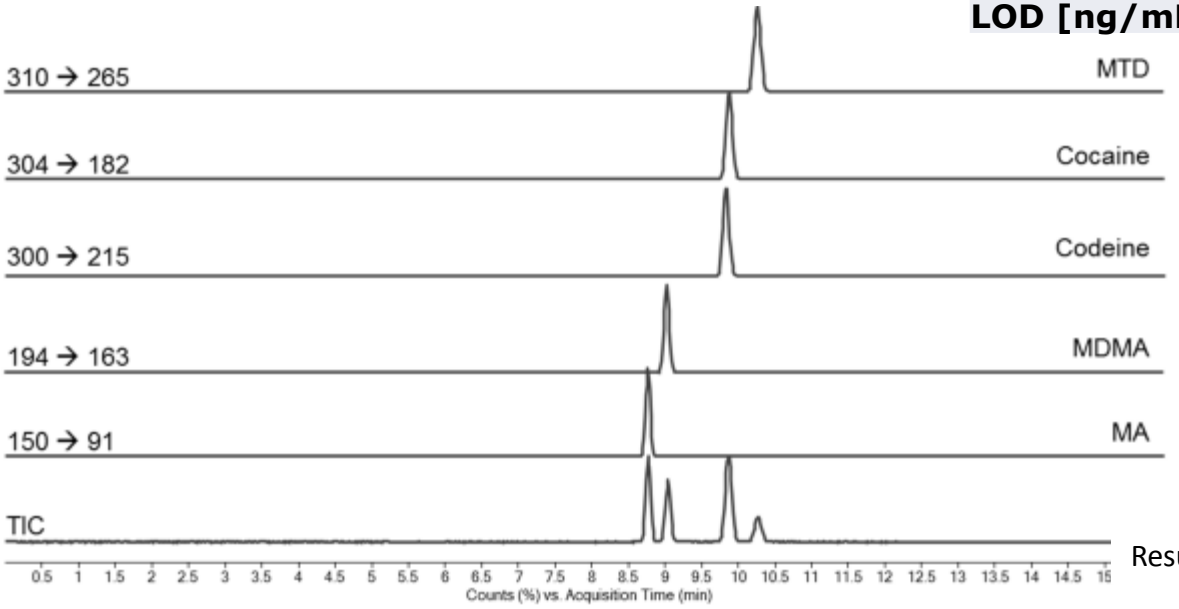
Current Status of CE-ESI/MS Coupling

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
LOD [ng/mL]	**0.5**	**0.5**	**5**	**2**	**50**

Sensitivity: ~ 2 - 200 nM concentration in sample

Results courtesy I. Kohler et al., University Geneva

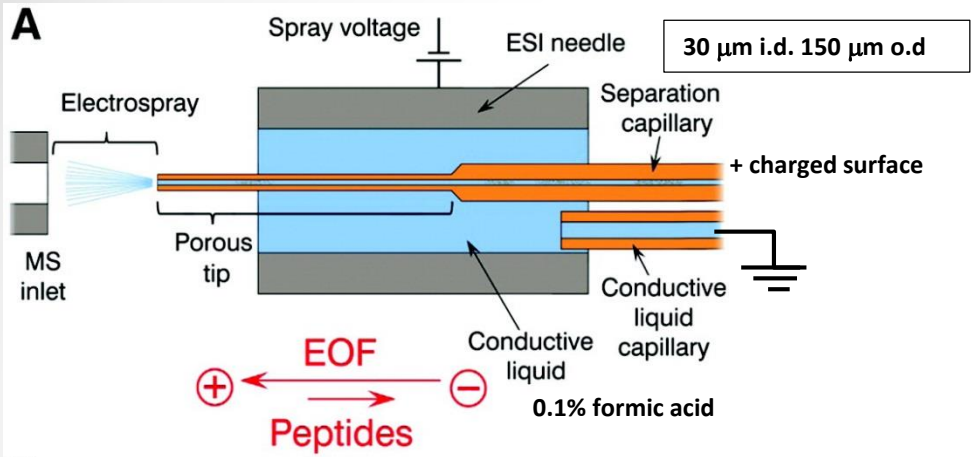
Recent Developments in CE-MS Coupling

- Porous tip approach*
- Micro flow-through vial**
- EOF driven sprayer***
- Sheath liquid contact approach****

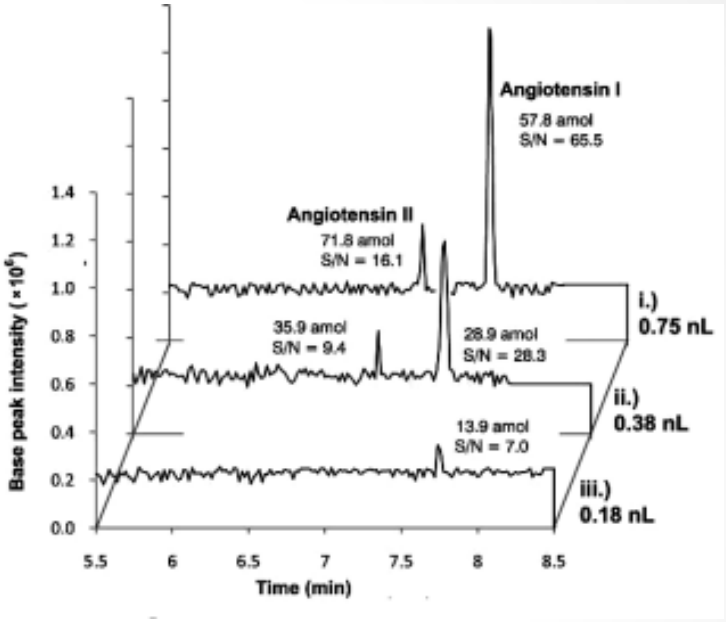
*M. Moini, Anal. Chem., **79**, 4241 (2007)
D.D.Y. Chen et al., Anal. Chem. **83, 4916 (2011)
***N. Dovichi et al., Rapid Comm. Mass Spec., **24**, 2554 (2010)
****R.D. Smith et al., Anal. Chem., **84**, 10395 (2012)

Recent Developments in CE-MS Coupling

Porous Tip Approach



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

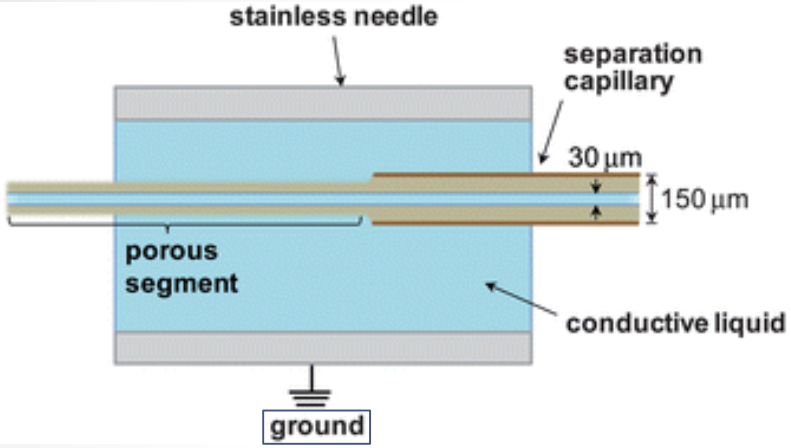


- Sensitivity: 10-20 nM AT1 concentration in sample
- >200 successive runs (pers. comm.)

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

Recent Developments in CE-MS Coupling

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



vs.

System: Agilent 6220 Accurate-Mass TOF LC/MS with Beckman Coulter PA800 plus CE and Agilent G7100 series CE respectively

Sample: Cationic metabolites, 2.3 and 2.6 nL injected resp.

BGE & Contact: 1 M formic acid

BGE & Sheath: 1 M formic acid, MeOH/Water with 0.1% hexakis

Capillaries 50 µm i.d. and 30 µm i.d. resp.

Sensitivity: 10-100 nM concentration in sample with porous tip

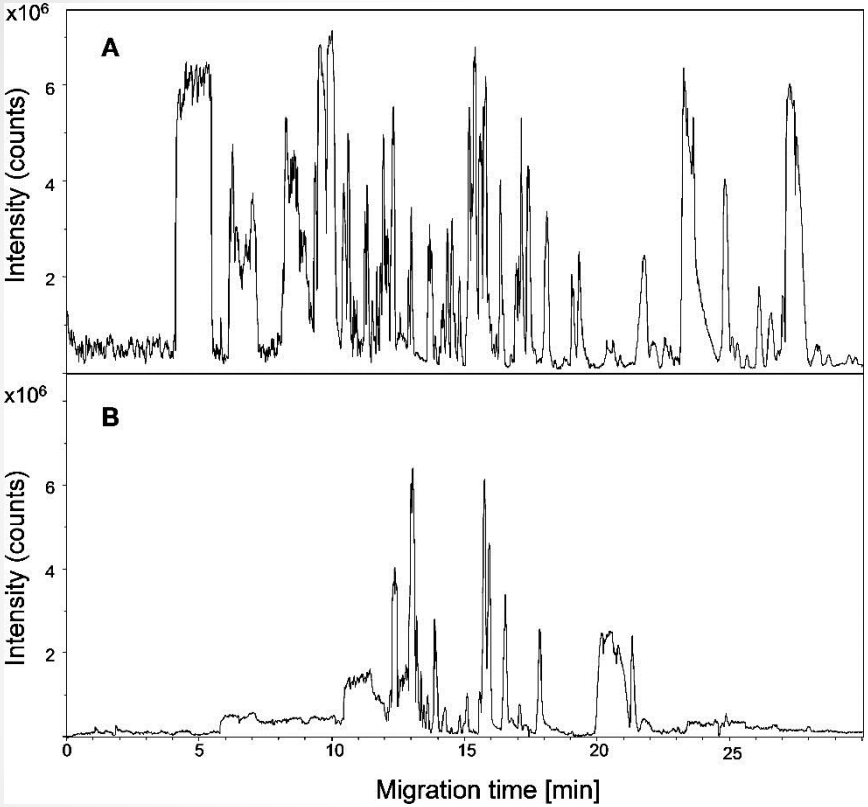
Relative: 0.2 – 20x sheathless/coaxial sheath flow

Robustness: 180 successive runs

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

Recent Developments in CE-MS Coupling

Comparison Coaxial Sheath Flow and Porous Tip (Ramautar et al.)



CE :	A: bare, 40 μm x 150 μm
	B: bare, 50 μm x 365 μm
BGE & Contact:	A: 10% HOAc, pH 2.2
BGE & Sheath:	B: Same, 0.1% HOAc/ Methanol 50/50, 4 μL/min
MS:	Bruker ToF → Tip grounded

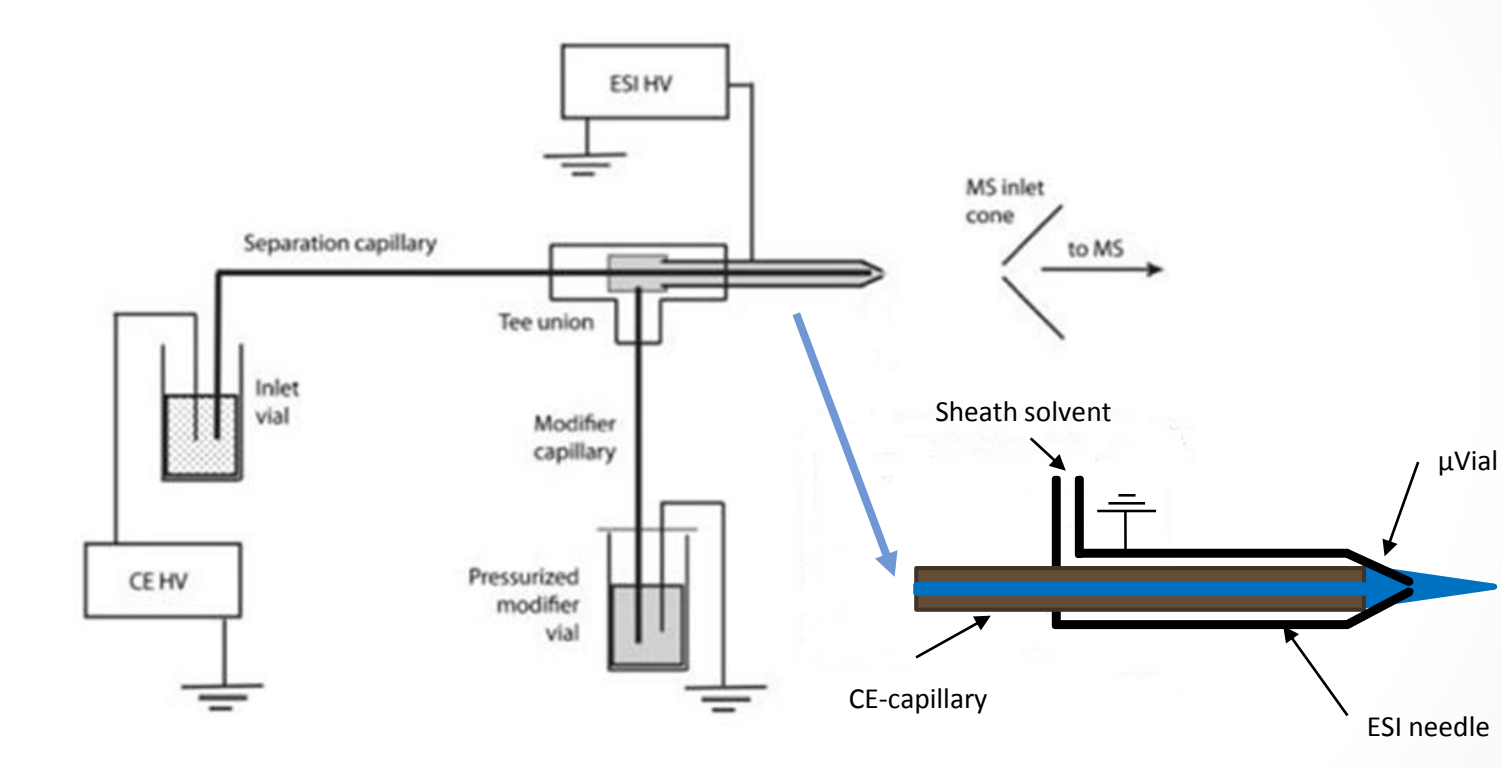
A. Base peak electropherogram (*m/z* 50–450) of human urine obtained with sheathless CE-MS using a porous tip sprayer. LOD 10-100 nM

B. Base peak electropherogram (*m/z* 50–450) of human urine obtained with CE-MS using a sheath-liquid interface. LOD 300-1000 nM

R. Ramautar et al., Anal. Chem., **84**, 885 (2012)

Recent Developments in CE-MS Coupling

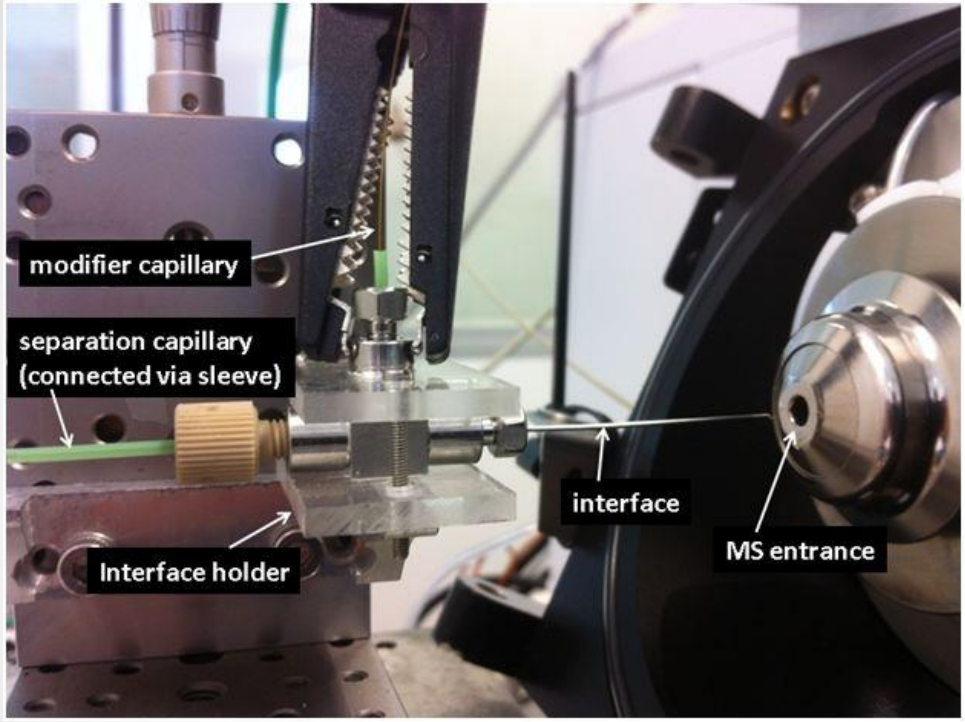
Micro Flow-Through Vial (D.D.Y. Chen et al.)



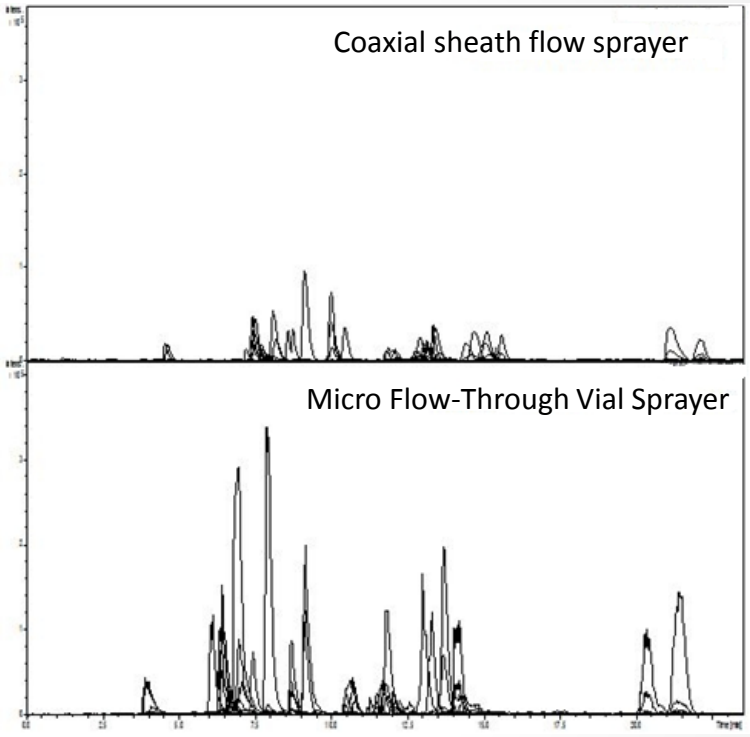
D.D.Y. Chen et al. Anal. Chem. **83, 4916 (2011)

Recent Developments in CE-MS Coupling

Micro Flow-Through Vial Practical Setup*



Very preliminary results

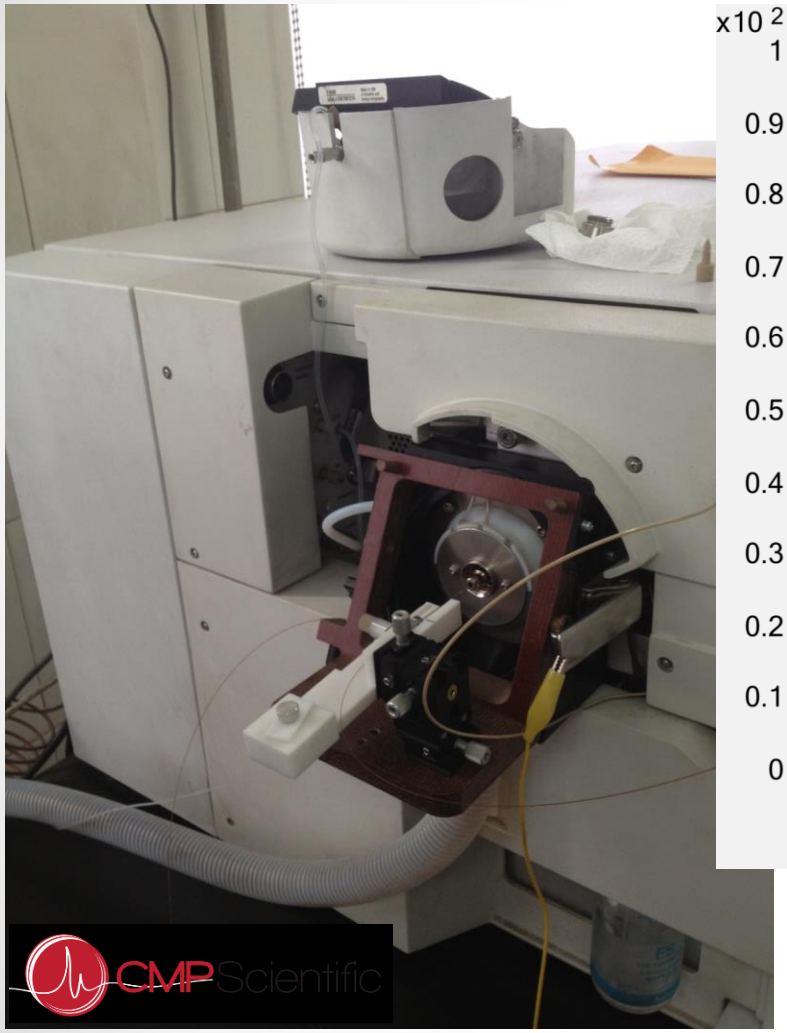


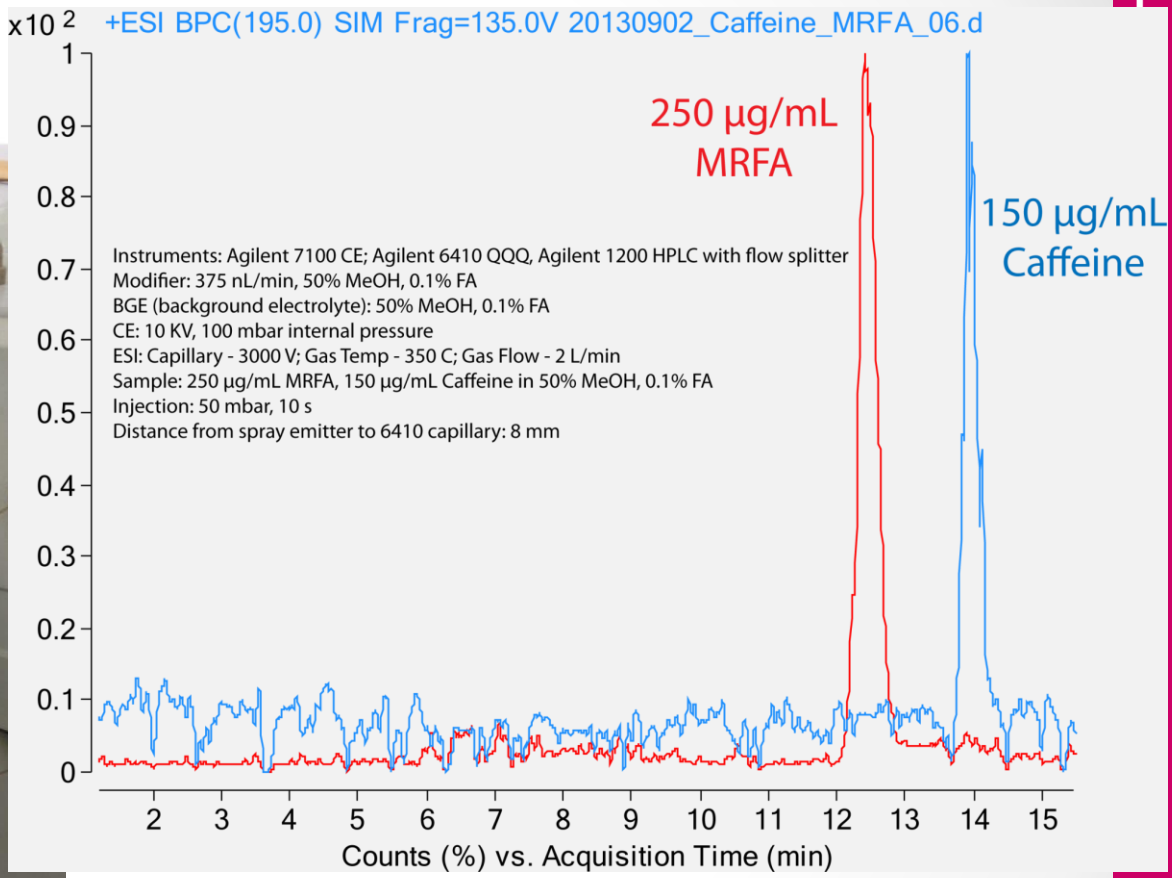
Sample: Cationic Metabolites from Human Metabolome Technologies
Sensitivity: 0.2 -4 μM
Improvement: 0.2 – 20x

*Results and Photo courtesy of Peter Lindenburg et al., Netherlands Metabolomics Center, Leiden, The Netherlands

Recent Developments in CE-MS Coupling

Micro Flow-Through Vial Practical Setup*



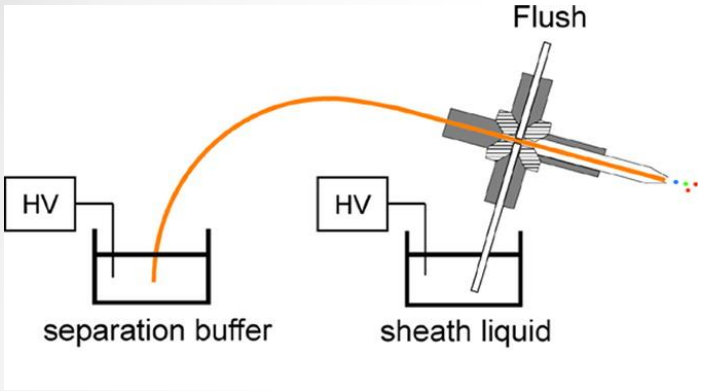


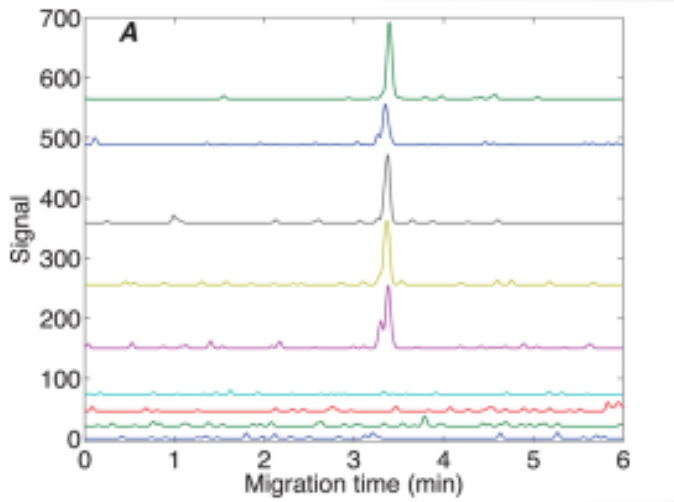
Sample: calibration mixture for electrospray ion sources (caffeine, MRFA (met-arg-phe-ala))
Sensitivity: approx. 5 µM

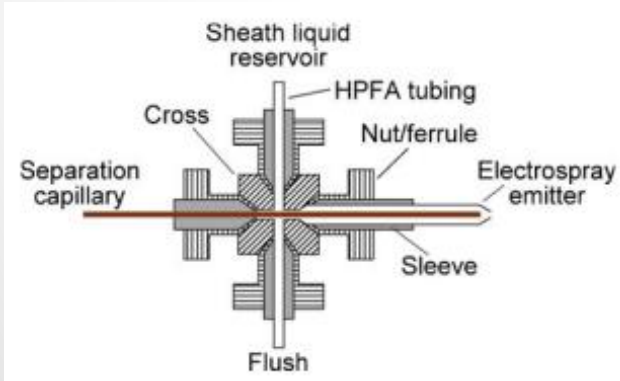
*Results and Photo courtesy of David Chen and CMP Scientific

Recent Developments in CE-MS Coupling

EOF Driven Sprayer (N. Dovichi et al)







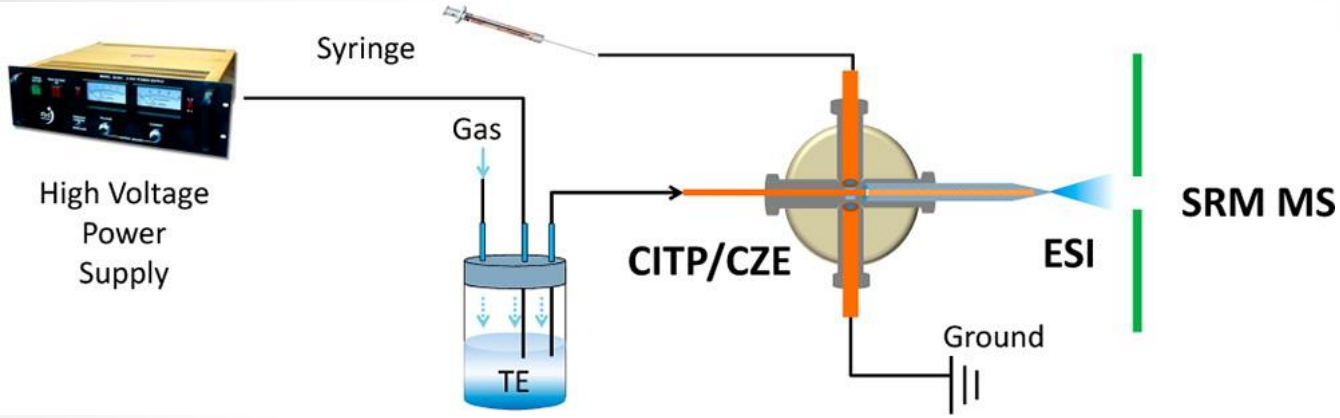
FS separation capillary 50x150 µm
Borosilicate emitter capillary 0.75x1 mm, orifice 5 µm
BGE 10 mM ammonium acetate, pH 5.5
Sheath solvent MeOH/0.1% formic acid
Sample: short peptides

Sensitivity: < 1 nM in sample concentration

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

Recent Developments in CE-MS Coupling

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



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

Summary | CE-MS Coupling

	Triple Tube	Porous Tip	Flow-through µVial	EOF Driven Tip	Smith approach
Sensitivity (LOD)	0.5 µM[b]/20 nM[a]	20 nM[b]	0.2 – 5 µM[c]	1 nM	50 pM[d]
Robustness/Reliability	xxx	xx	xx[c]	?	?
Ease of Use	xxx	xx	xx	?	?
Standard Capillaries?	YES	NO[e]	YES	NO[e]	NO[e]

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 and possible
d. In combination with cITP
e. special 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 MS, CE will give higher peaks than in HPLC!
  - 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 μVial-flow through IF seem an promising pathway towards CE-ESI/MS.
- Commercialization (affordable) will be the key for success of new sheathless CE-ESI/MS coupling methods

Acknowledgements

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- Herbert Lindner, Innsbruck Medical University
- Peter Lindenburg, Rawi Ramautar, Thomas Hankemeier Netherlands Metabolomics Center, Leiden University
- David Chen, University of British Columbia, Canada
- CMP Scientific, Hoboken, USA