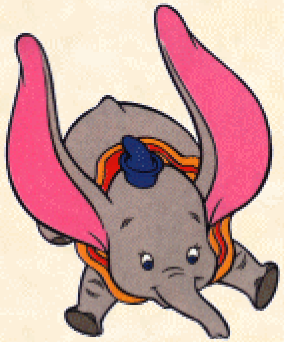
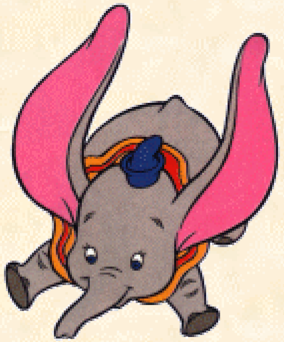
Fundamentals of Electrospray Ionization Symposium


pre-electrospray

Support and Thanks


pre-electrospray

Analytical Chemistry Division – ACS

ThermoFinnigan

Waters/Micromass   Applied Biosystems   IonSpec

Invited Speakers

The Electrochemistry of Electrospray

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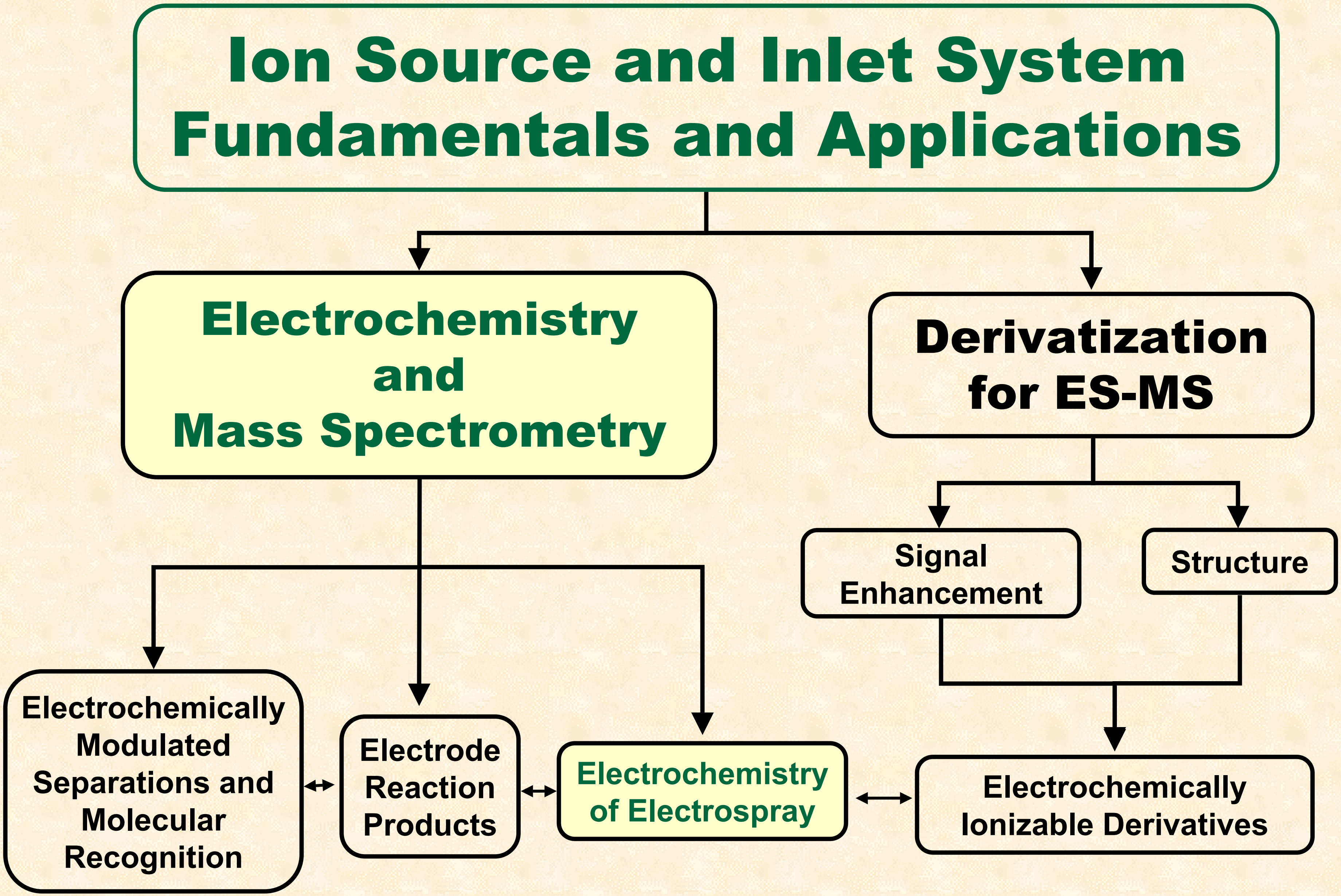
Fundamentals of Electrospray Ionization Symposium
ACS National Meeting, New Orleans, LA
March 25, 2003

http://www.ornl.gov/csd/Research_areas/obms_rd.html

Funding
Office of Basic Energy Sciences – DOE
SCIEX - Cooperative Research and Development Agreement


Ion Source and Inlet System
Fundamentals and Applications
Electrochemistry and Mass Spectrometry
Derivatization for ES-MS
Signal Enhancement
Structure
Electrochemically Modulated Separations and Molecular Recognition
Electrode Reaction Products
Electrochemistry of Electrospray
Electrochemically Ionizable Derivatives

Electrochemistry/Mass Spectrometry
Electrochemistry of Electrospray

Built-in Capabilities:

- *Controlled-current electrochemistry (CCE) inherent to the operation of an electrospray ion source*

Add-on Possibilities:

- Discrete electrochemical cells
  coupled on-line via the electrospray ion source
    - controlled-potential electrochemistry (CPE)
    - *intertwined CCE/CPE (floated 3-electrode cell)*

Electrochemistry of Electrospray

Analytical Issues

Minimize Analyte Involvement

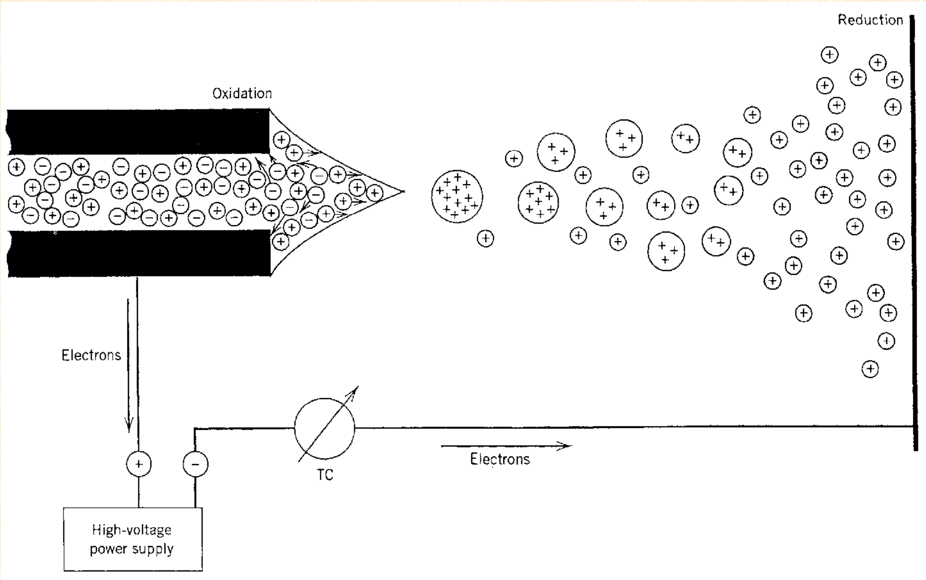
- avoid confusion in the analysis of unknowns - change in mass or charge
- preserve initial solution state of analyte
- avoid distribution of charge among different ionic species

Maximize Analyte Involvement

- electrochemical ionization
- create novel ionic species
- study analyte redox chemistry
- perform electrosynthesis

The Electrochemistry of Electrospray

Fundamental understanding of the ES ion formation process will enhance and expand the considerable analytical impact of this process



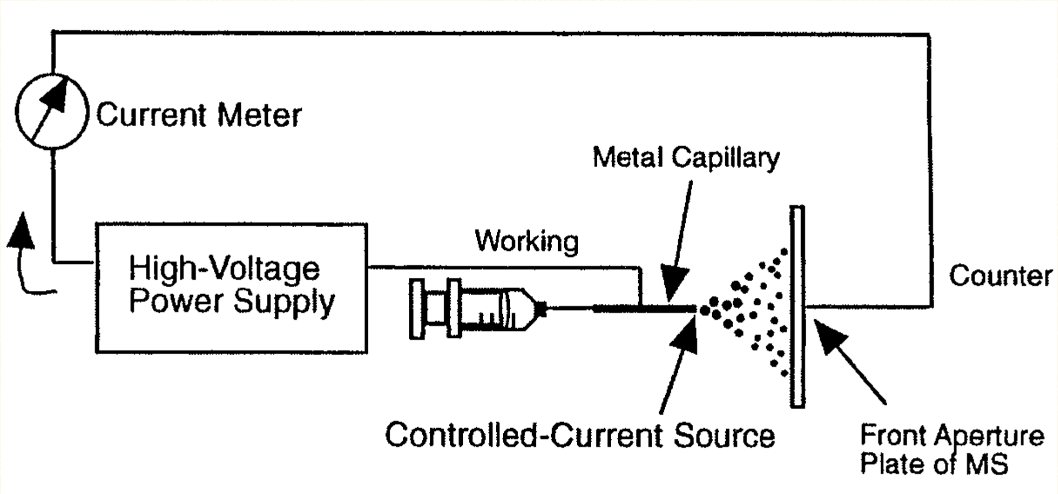
Blades, Ikonomou, Kebarle: Analytical Chemistry 1991, 63, 2109

Van Berkel and Zhou: Analytical Chemistry 1995, 67, 2916

Our investigations of the electrospray electrochemistry are driven by a quest to learn the answers to several key questions:

- Does the varied electrochemistry that occurs at the emitter electrode affect the gas-phase ion abundances observed in the ES mass spectra?
- Can various operational parameters and ES emitter geometries and materials be used to either minimize or maximize (or otherwise control) direct or indirect analyte involvement in the ES electrochemical processes (heterogeneous and homogeneous electron-transfer chemistry, respectively)?
- Does the electrochemistry play a role in the identity and absolute magnitude of chemical noise in the ES mass spectra?

The Electrochemistry of Electrospray



- Ions must be present in the solution
- Ions transport charge between the electrodes by means of the charged droplets
- Electrochemical reactions at the ES emitter and counter electrodes complete the circuit

- Redox reactions may involve the metal emitter and/or solution species
- Electrochemical behavior that of two electrode controlled-current cell
- The interfacial potential at the ES emitter electrode is a *complex function* of the ES current, the effective electrode area, the relative redox potentials and concentrations of the various electroactive species present, and the solution flow rate
- The extent to which one or more reactions occur is influenced by the interfacial potential, ES current, species present, solution flow rate, and other factors affecting mass transport to the electrode surface

Electrochemistry of Electrospray

$$[\mathrm{Q}] = \frac{i_{\mathrm{ES}}}{F v_{\mathrm{f}}} = \sum_j (n_j [\mathrm{EP}]_j)$$

The greatest effect/influence of the ES electrochemistry on the appearance of an ES mass spectrum is to be expected at very low solution flow rate

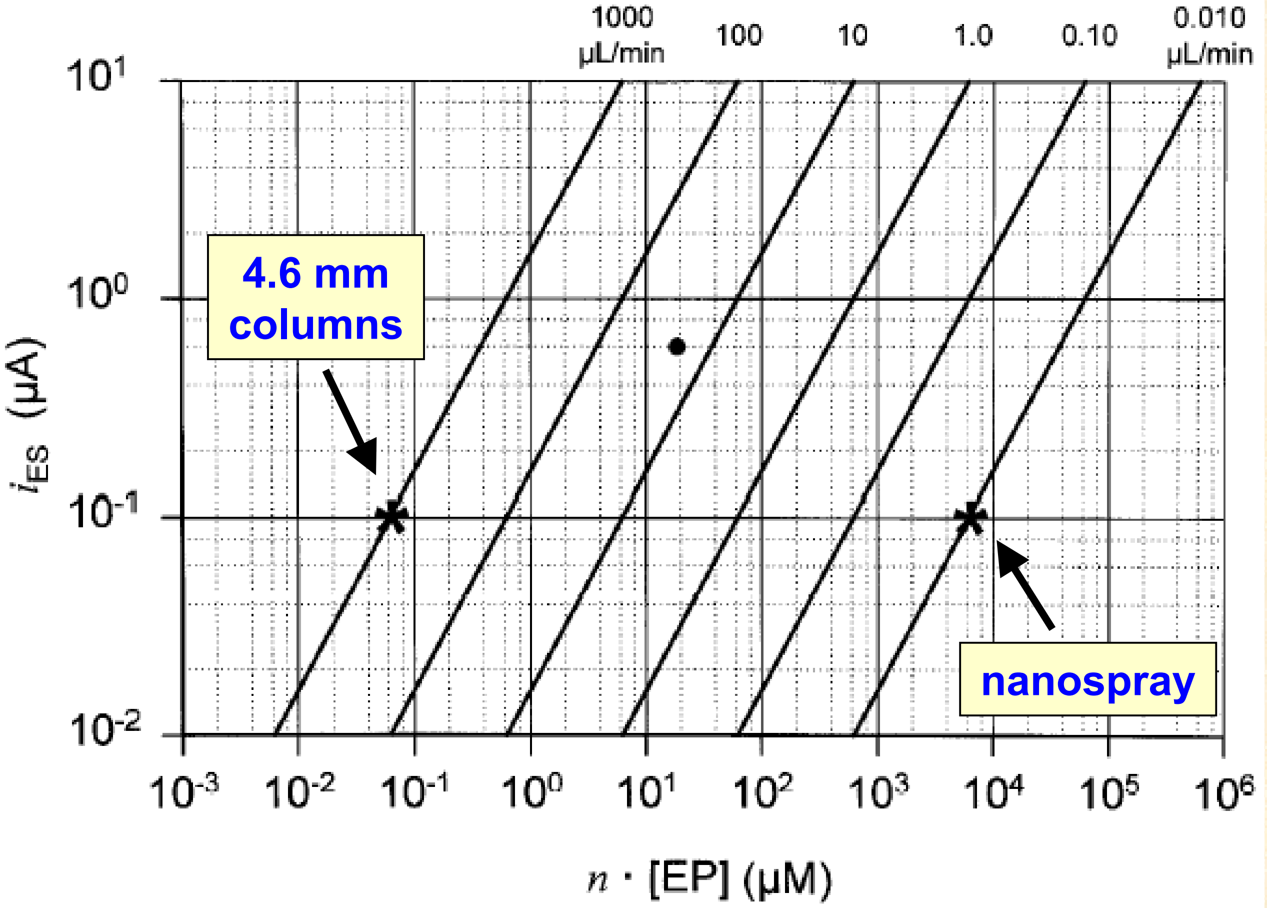
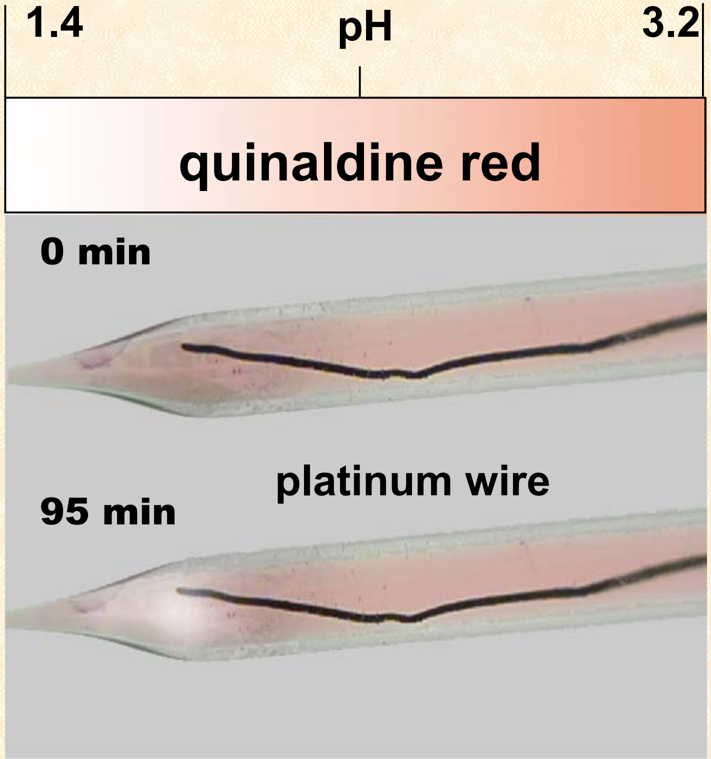
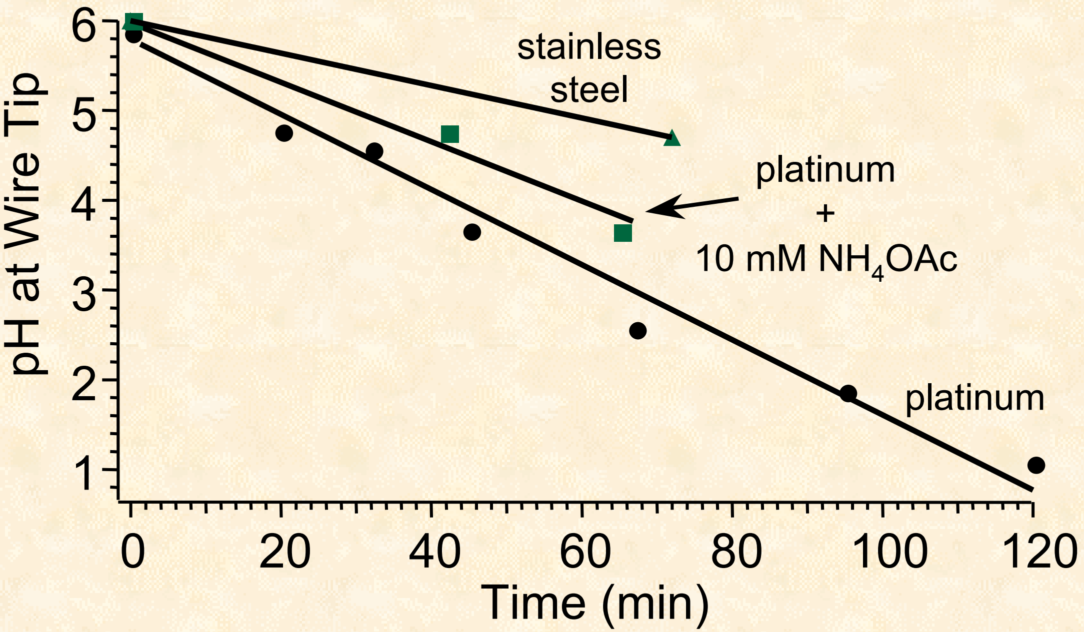


Figure 1. Theoretical plots of the concentration of the electrolytic products, [EP], added to or removed from the solution electrosprayed via the electrolytic processes in the ES emitter as a function of solution flow-rate through the emitter. Plots were calculated using Eqn (1) assuming that only one electrolytic reaction occurred. Solid circle: $i_{ES} \approx 0.6$ µA, $v_f = 20$ µl min^{-1}, $n = 1$ and [EP] ≈ 19 µM, which are the values for these parameters for the experiments in Fig. 3. The asterisks mark both [EP] = 0.062 µM and 6.2 mM for the ESMS conditions described in the text.

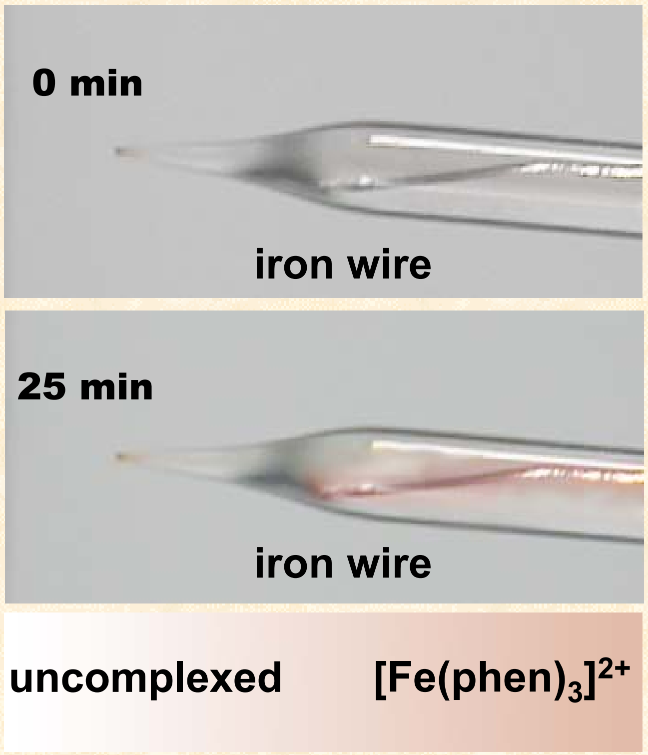
Electrochemical Processes in a Wire-in-a-Capillary Bulk-Loaded, Nano-Electrospray Emitter

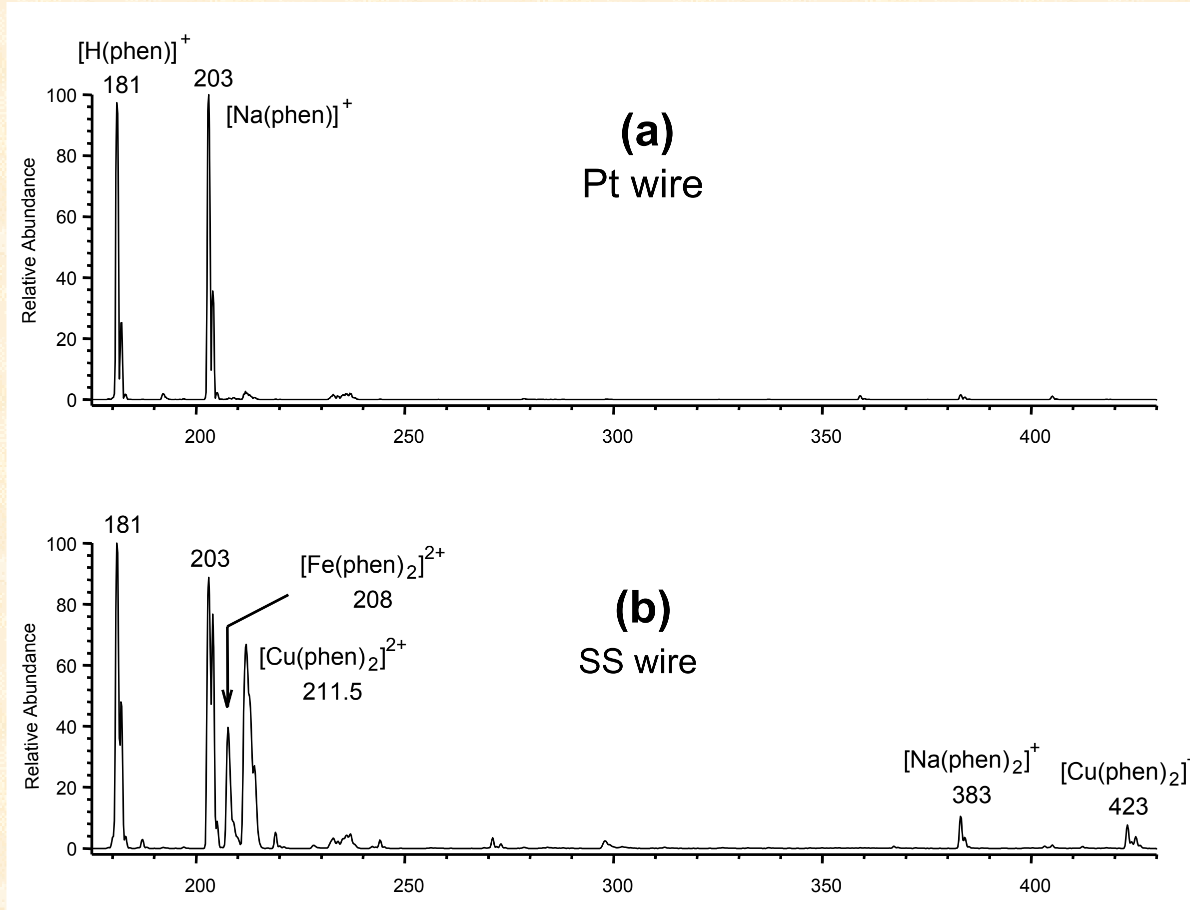




- oxidation of water at electrode tip may change pH by several pH units over the time of a typical experiment
- products of electrochemical reactions can ‘buildup” in the capillary
- magnitude of change may be mitigated by nature of electrode material and solution composition

Electrochemical Processes in a Wire-in-a-Capillary Bulk-Loaded, Nano-Electrospray Emitter





- corrosion of metal wire electrode adds metal ions to solution
- corrosion may occur a substantial distance upstream
- corrosion might be a source of metal ions for metal-ligand studies

Analyte Oxidation in a Metal Capillary Emitter Electrode

Minimize Analyte Oxidation

Why?

- avoid confusion in the analysis of unknowns - change in mass or charge
- preserve initial solution state of analyte
- avoid distribution of charge among different ionic species

How?

- sacrificial electrode/redox buffer
- high solution flow rate ($\geq$30 💻L/min)
- short electrode (<<1 mm)
- low solution conductivity (10^{-4} S/m)
- low electrospray voltage drop ($\leq$4kV)

Maximize Analyte Oxidation

Why?

- electrochemical ionization
- create novel ionic species
- study analyte redox chemistry
- perform electrosynthesis

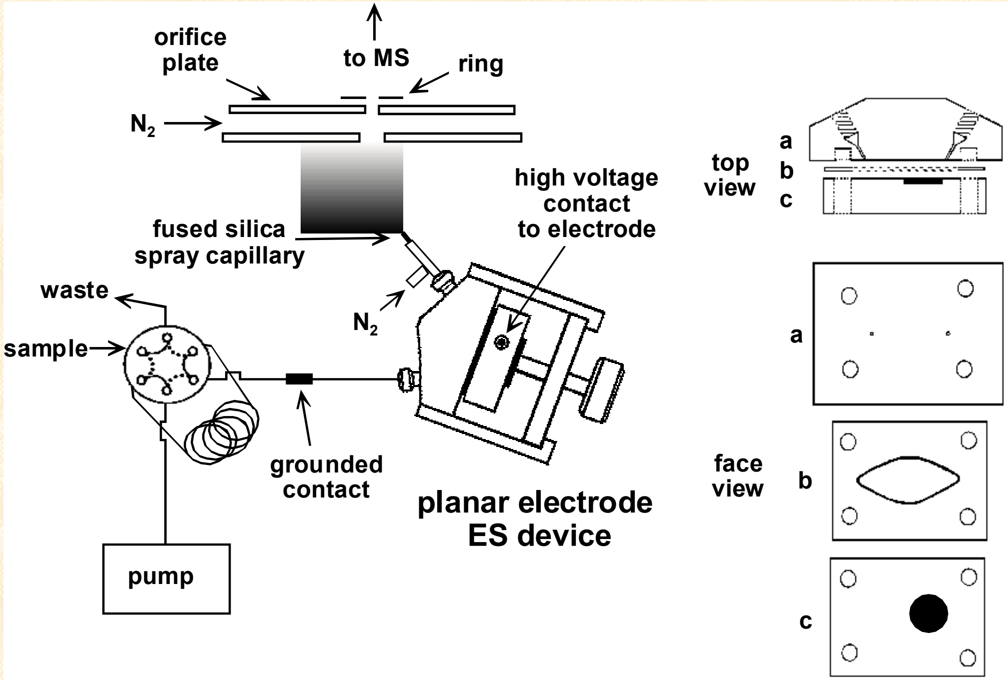
How?

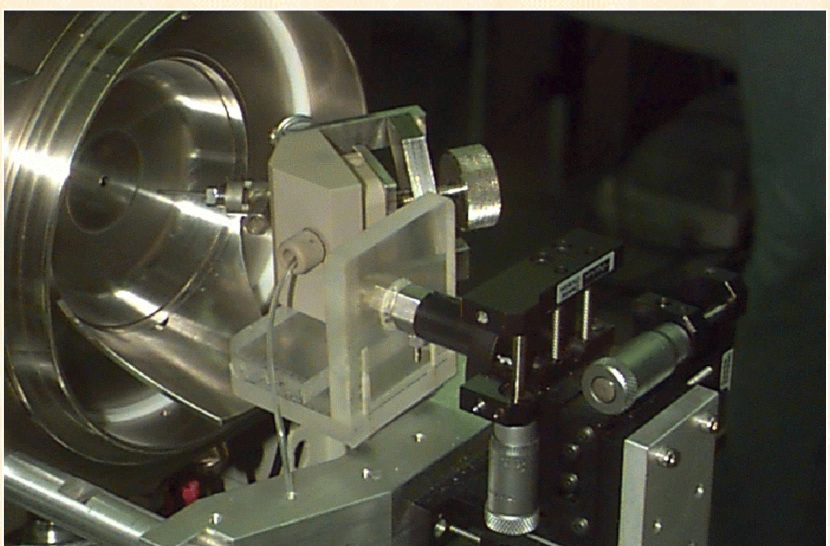
- inert electrode (platinum)
- low solution flow rate ($\leq$2.5 💻L/min)
- long electrode (>several mm)
- high solution conductivity (>>10^{-4} S/m)
- high electrospray voltage drop (>4 kV)

The Electrochemistry of Electrospray:
Where are we and what's left to be done?

- The electrochemistry ongoing in an ES ion source can be a major influence on the nature and abundance of the ions observed in an ES mass spectrum - compound and analysis conditions dependent
- General electrochemical principles of operation established
- Analytical effects and reaction control most studied in tubular electrode systems
- Several areas associated with ES electrochemistry lack investigation:
    - electrode material (various metals, carbons, conductive polymers)
    - electrode geometry and area of contact with solution (mass transport, current density)
    - Intertwining of controlled current and controlled potential cells (floated 3-electrode cell)
    - homogenous solution reactions (coulometric titrations)
    - computational simulations
    - electrochemistry in negative ion mode

Thin-Channel Electrode Electrospray Emitter





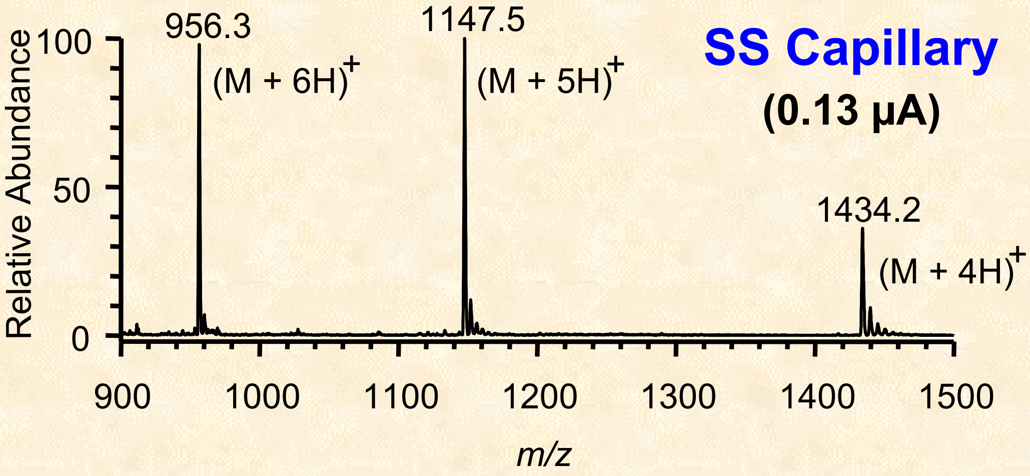
Facilitates the study and utilization of key parameters that influence the electrochemical processes:

Easily and quickly -

- **change electrode material (SS, Pt, Au, Ag, Zn, Cu, Ni, etc.) and area**
- **alter flow dynamics through change in electrode area, thin-layer channel thickness, or flow rate**
- **use as electrodes materials not available in tubular form (glassy carbon)**
- **make and use modified electrode surfaces (conductive polymers, catalytic electrodes)**

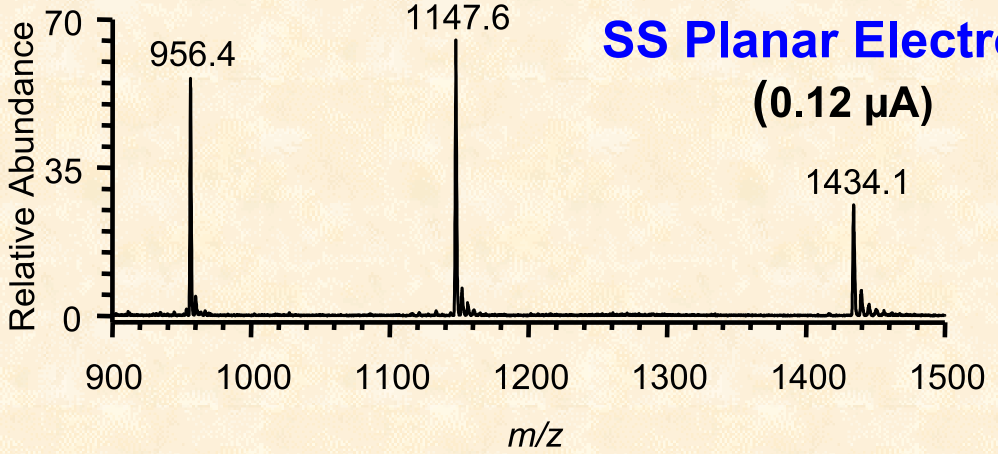
Thin-Channel Electrode Electrospray Emitter
“non-electroactive” analytes

1.7 μM bovine insulin, 1/1 (v/v) water/acetonitrile
0.1% by vol HOAc, 10 μL/min, 4.5 kV



SS Capillary
(0.13 μA)

TurboIonSpray™ source with a 304 stainless steel capillary emitter electrode

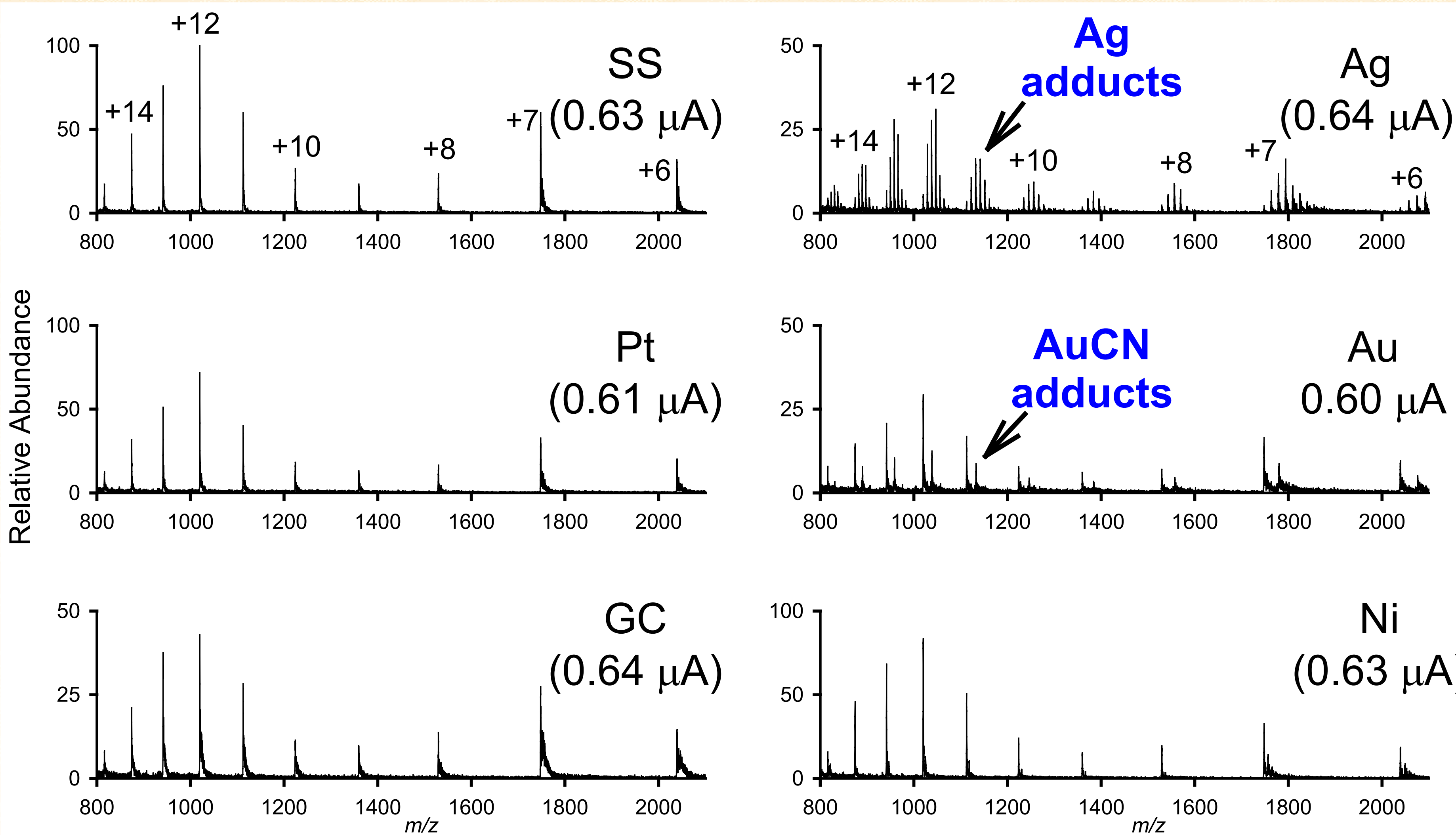


SS Planar Electrode
(0.12 μA)

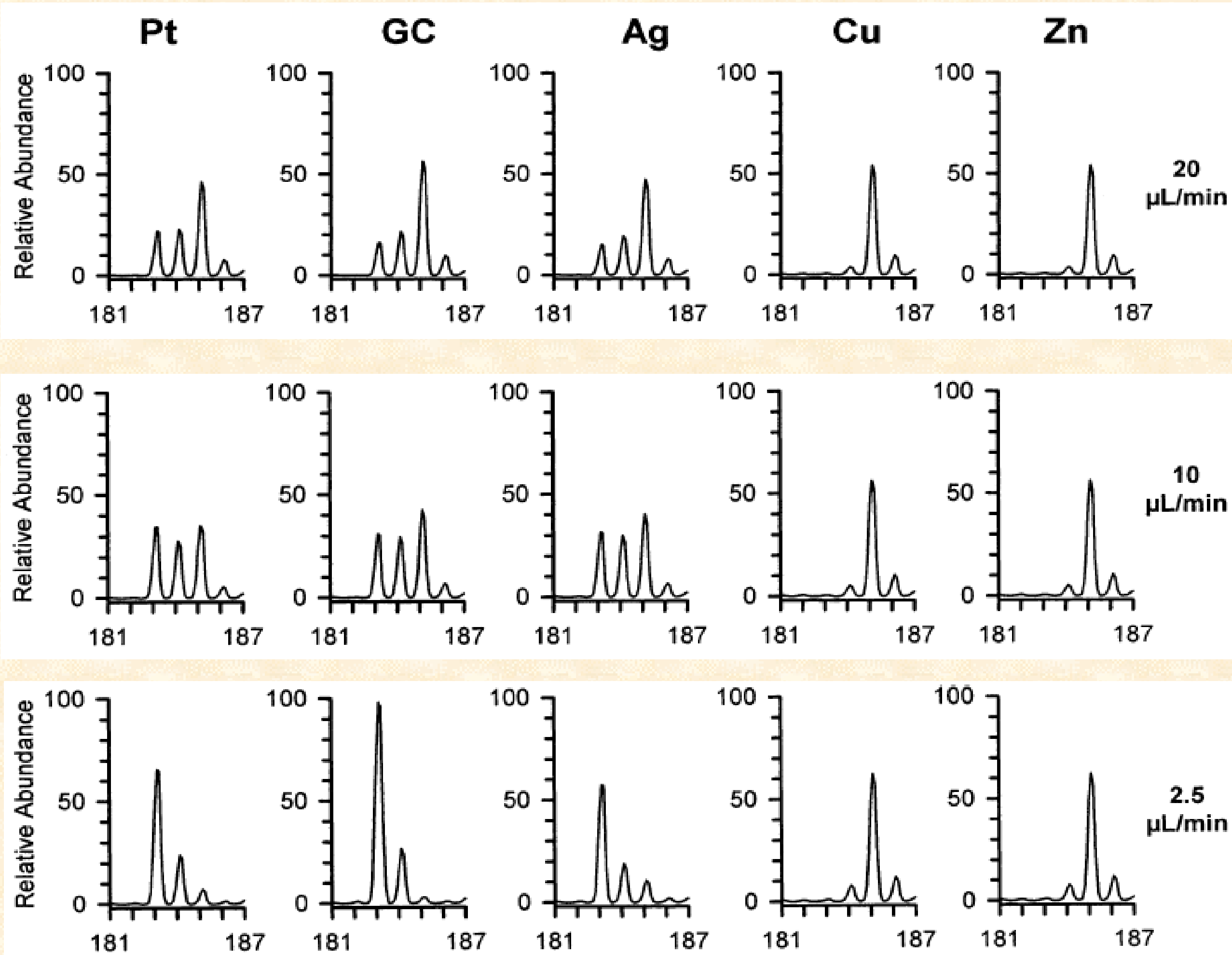
Thin-channel electrode emitter device with a 6.0 mm dia., 316L stainless steel disk electrode (10 μm spacing gasket)

Thin-Channel Electrode Electrospray Emitter
“non-electroactive” analytes

1.0 μM Cytochrome *c*, 1/1 (v/v) water/acetonitrile
5 mM NH_4OAc, 0.75% by vol HOAc, 2.5 μL/min, 4.5 kV

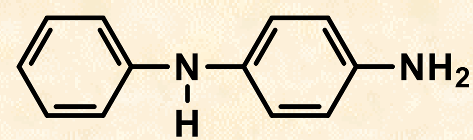


Thin-Channel Electrode Electrospray Emitter
"electroactive" analytes

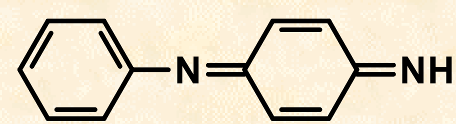


20 µM
***N*-phenyl-1,4-phenylenediamine**
1/1 (v/v) water/methanol
5 mM NH_4OAc, 0.75% by vol HOAc, 2.5 µL/min, 4.5 kV

Red = Ox + $2H^+$ + $2e^-$



$(Red + H)^+$ = *m/z* 185



$(Ox + H)^+$ = *m/z* 183

Thin-Channel Electrode Electrospray Emitter

“electroactive” analytes

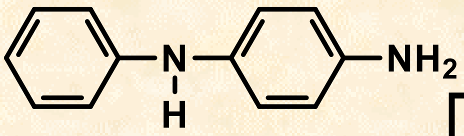
Table 1. Calculated ES Current Required for 100% Oxidation of 1 to 1a and the Measured i_{ES} Values for the Various Experiments Shown in Figure 3

flow rate (μL/min)	i_{ES} required for 100% oxidation (μA)[a]	i_{ES} measured (μA)[b]				
		Pt	GC	Ag	Cu	Zn
1.5	0.0965	*0.19*	*0.25*	*0.24*	*0.24*	*0.24*
2.5	0.16	*0.20*	*0.24*	*0.24*	*0.24*	*0.24*
5.0	0.32	0.22	0.26	0.26	0.24	0.26
10	0.64	0.25	0.26	0.28	0.26	0.29
15	0.965	0.25	0.28	0.30	0.28	0.30
20	1.3	0.26	0.28	0.30	0.30	0.31

[a] i_{ES} required = $nFvC_A$, where i_{ES} required is the current magnitude for oxidation of the molar equivalent of the analyte flowing through the emitter, n is the molar equivalent of electrons involved in the redox reaction of **1** to **1a** (n = 2), F is the Faraday constant (9.65×10^4 C/mol), v is the volumetric flow rate through the emitter, and C_A is the bulk solution concentration of the analyte. [b] Values in italic type indicate i_{ES} measured > i_{ES} required. Values in roman type indicate i_{ES} measured < i_{ES} required.

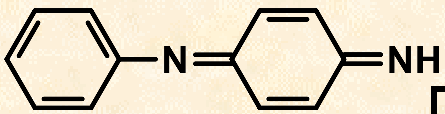
20 μM
***N*-phenyl-1,4-phenylenediamine**
1/1 (v/v) water/methanol
5 mM NH_4OAc, 0.75% by vol HOAc, 2.5 μL/min, 4.5 kV

Red = Ox + $2H^+$ + $2e^-$



1

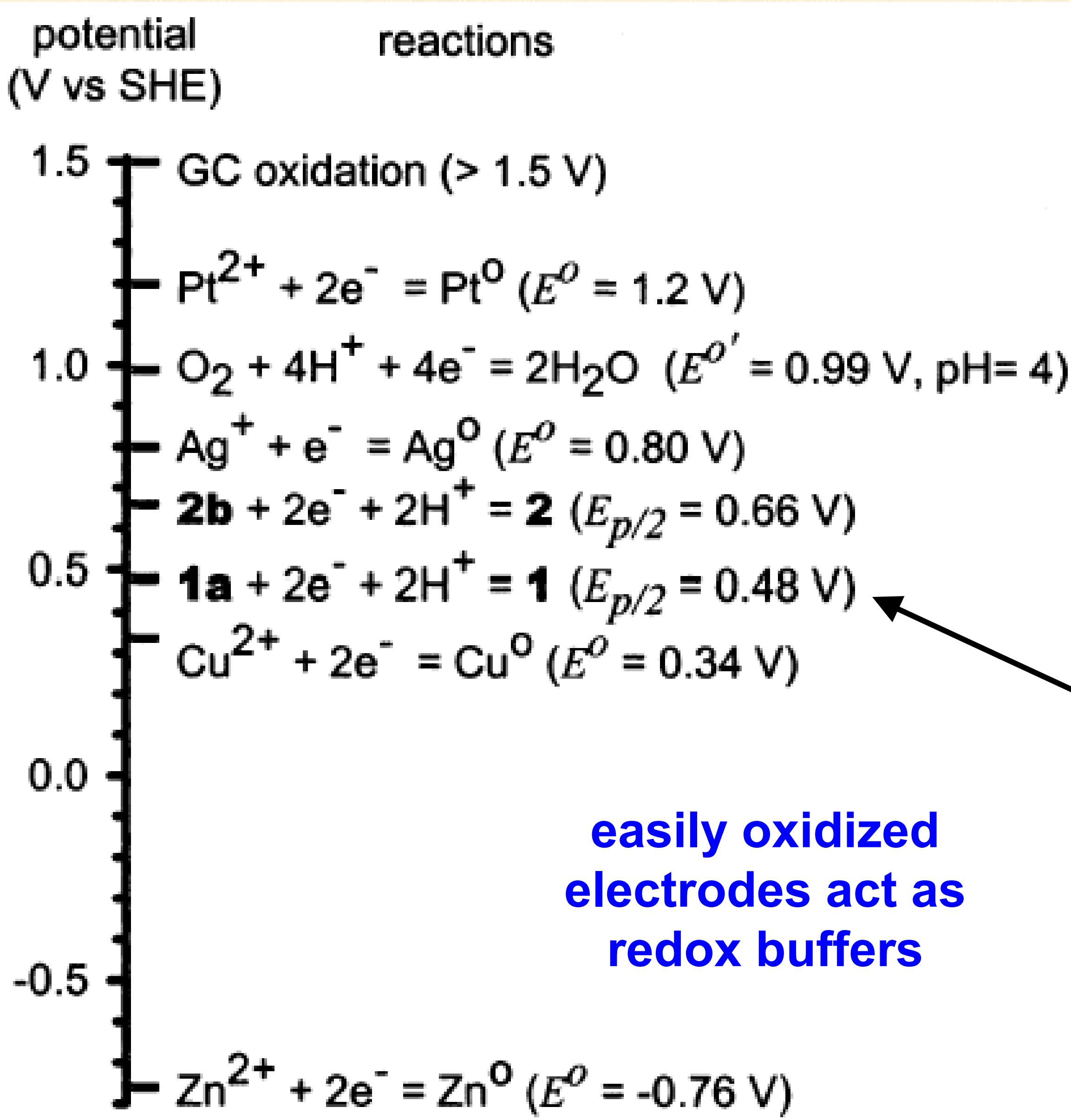
(Red + H)$^+$ = *m/z* 185



1a

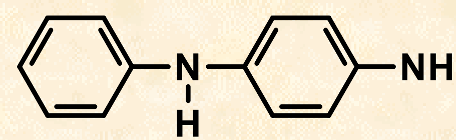
(Ox + H)$^+$ = *m/z* 183

Thin-Channel Electrode Electrospray Emitter
“electroactive” analytes



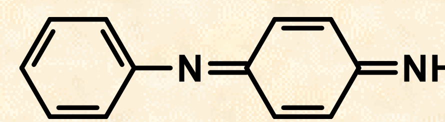
20 μM
N-phenyl-1,4-phenylenediamine
1/1 (v/v) water/methanol
5 mM NH_4OAc, 0.75% by vol HOAc, 2.5 μL/min, 4.5 kV

Red = Ox + $2H^+$ + $2e^-$



1

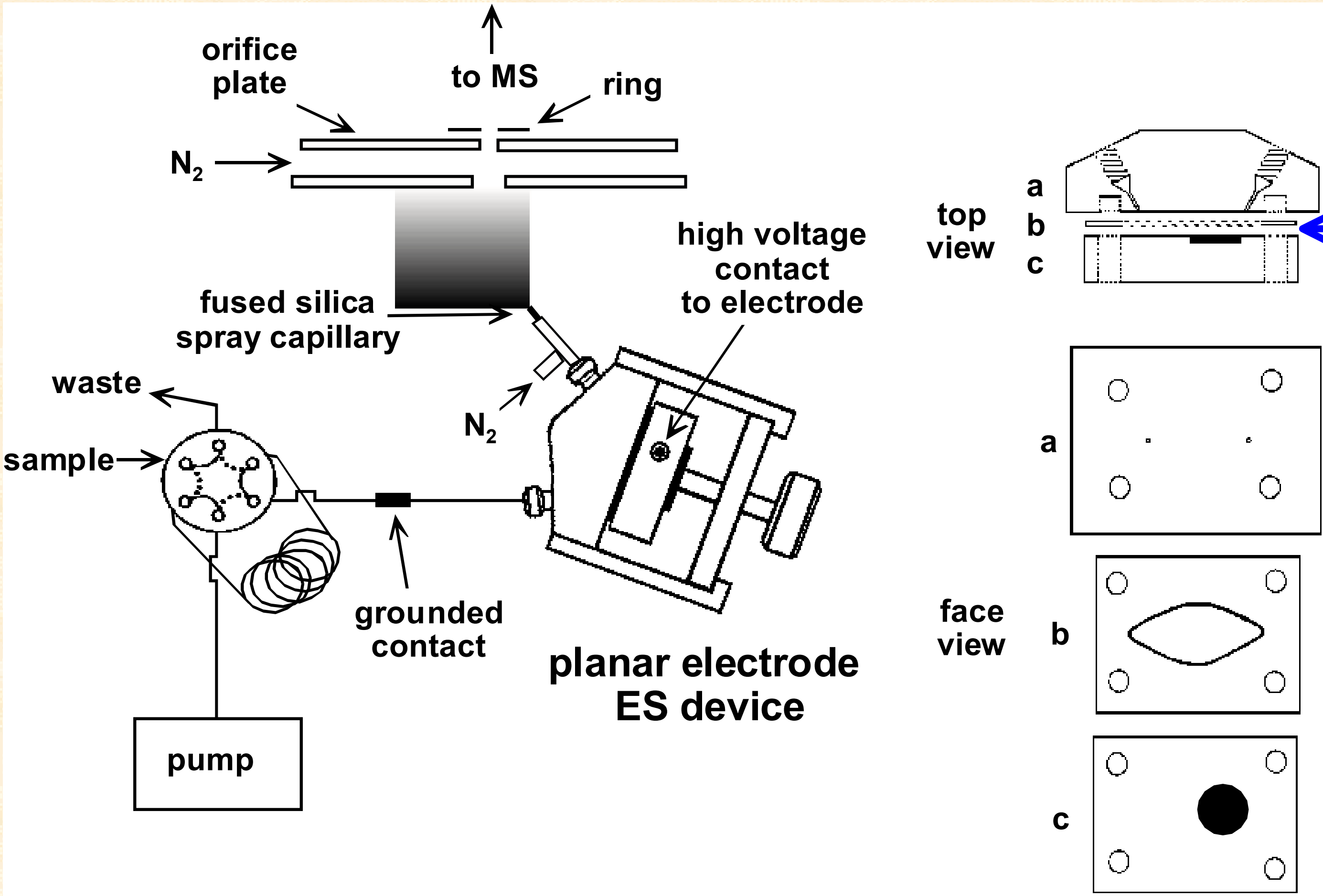
(Red + H)$^+$ = *m/z* 185



1a

(Ox + H)$^+$ = *m/z* 183

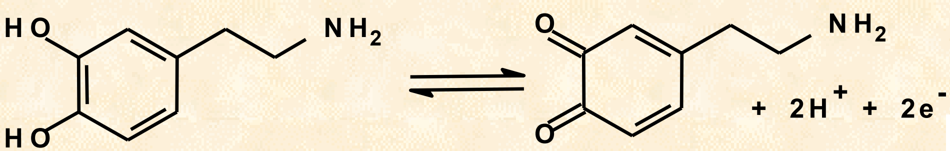
Thin-Channel Electrode Electrospray Emitter
“electroactive” analytes



- **Insert 5000 Da molecular mass cutoff membrane on electrode side of spacing gasket**
- **Membrane permits ion transport**
- **Membrane inhibits analyte transport to emitter electrode**

Thin-Channel Electrode Electrospray Emitter
“electroactive” analytes

20 μM Dopamine
1/1 (v/v) water/methanol
5 mM NH_4OAc, 0.75% by vol HOAc
6.0 mm GC electrode, 4.5 kV
127 μm spacing gasket

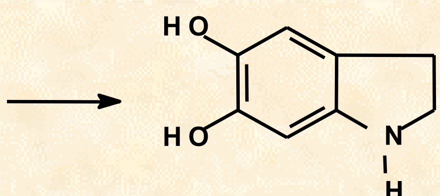


2 **2a**

$(\mathbf{2} + H)^+ = m/z\ 154$ $(\mathbf{2a} + H)^+ = m/z\ 152$

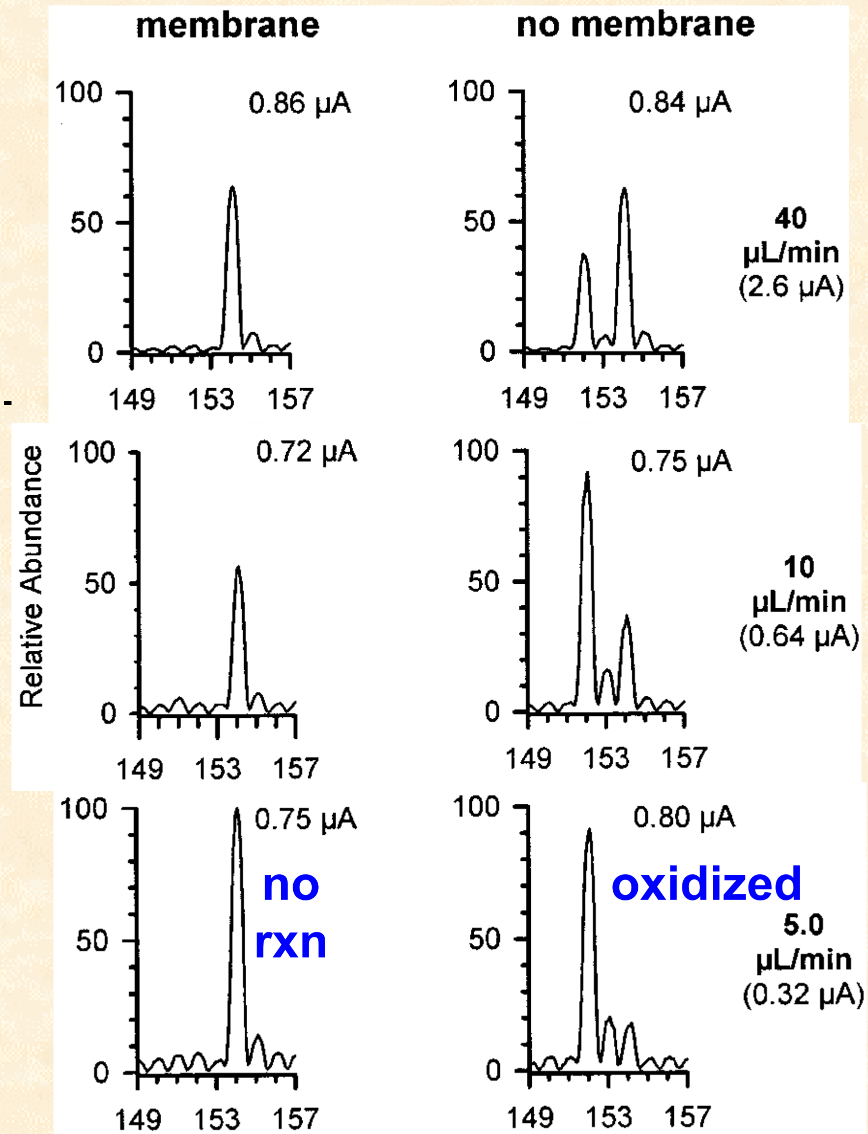
$\mathbf{2b} + 2e^- + 2H^+ = \mathbf{2}\ (E_{p/2} = 0.66\ V)$

2a



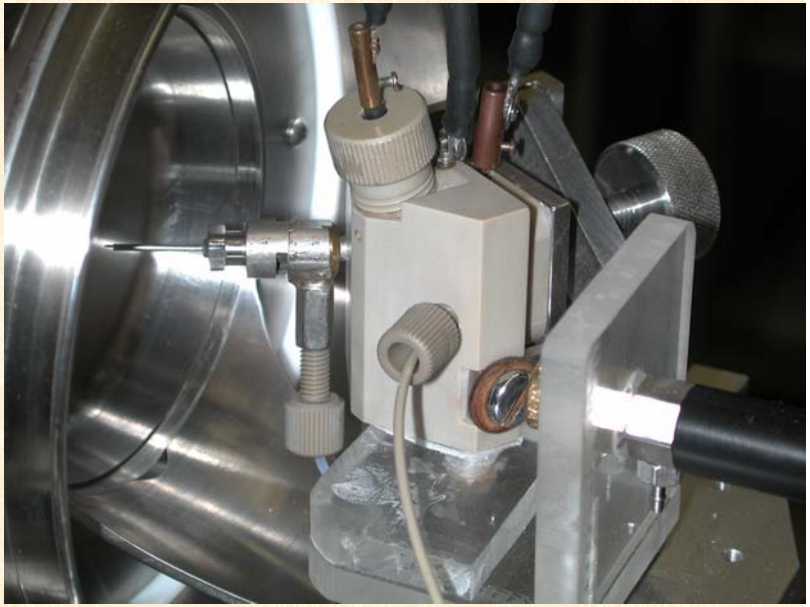
2b

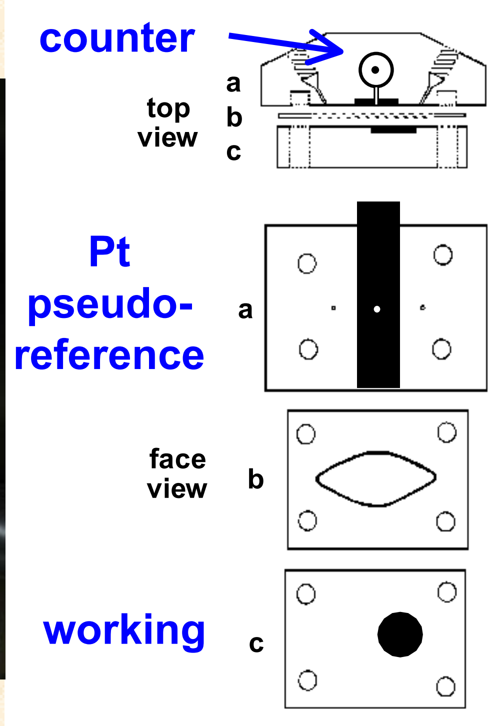
$(\mathbf{2b} + H)^+ = m/z\ 152$



Thin-Channel Electrode Electrospray Emitter

Floated 3-Electrode Cell



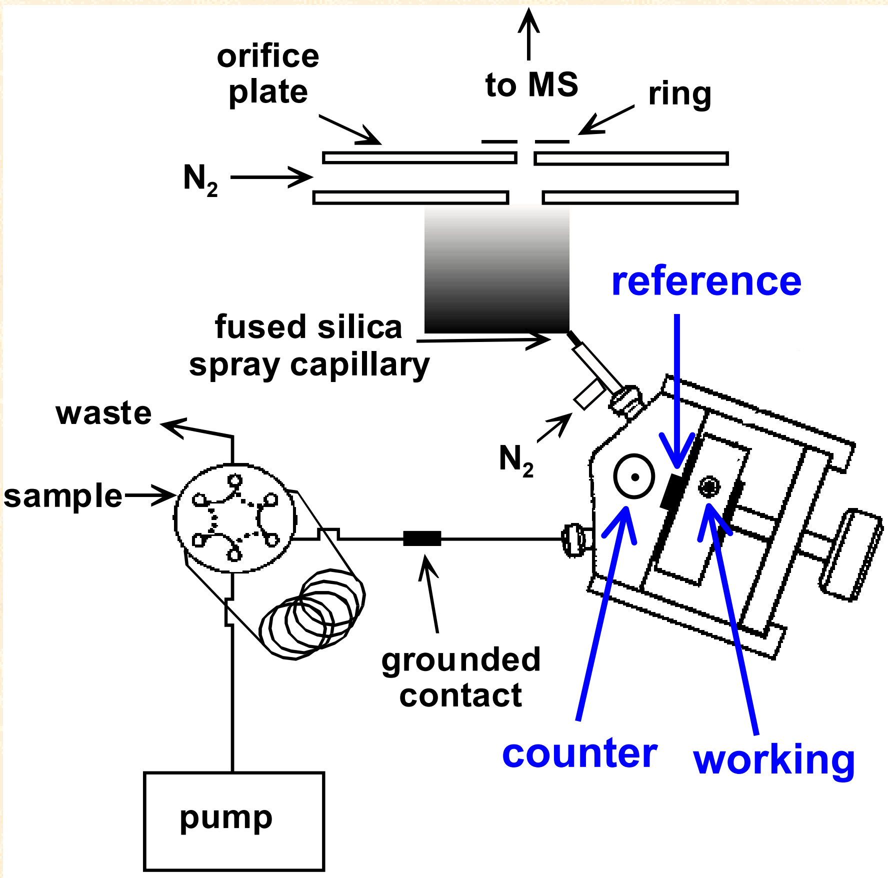


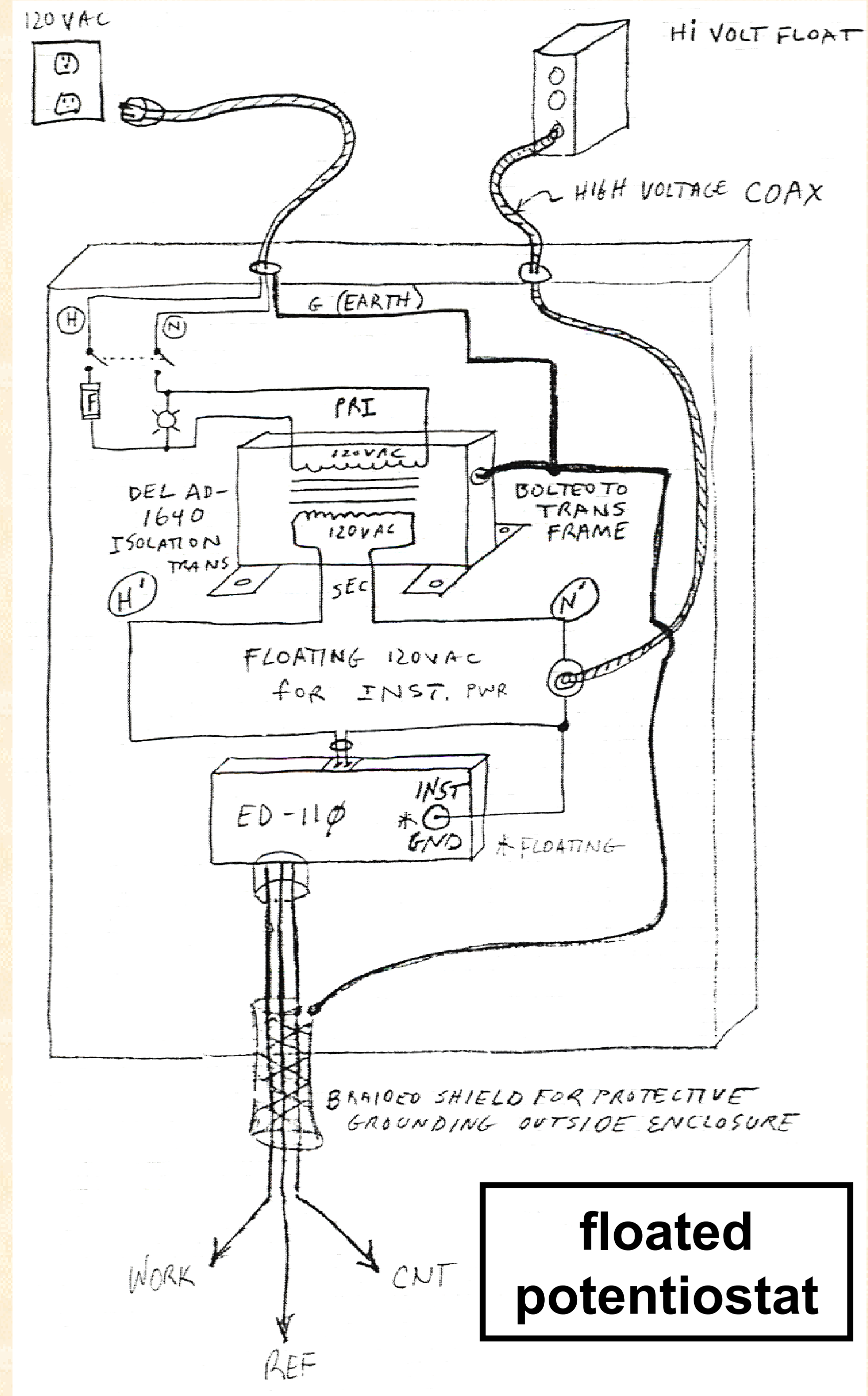
Three electrode system (working, counter, reference) with a suitably configured potentiostat, adds another discrete electrochemical cell into the circuit, floating at the electrospray high voltage

- Determine the fundamental and analytical implications (for ES and the electrochemistry) of interweaving the controlled-current electrochemical process of ES and the controlled-potential process of the discrete electrochemical cell
- Carry out electrochemical experiments with a very fast response time (< 1.0 s), low-dead volume electrochemical cell system - investigation of short-lived intermediates, highly efficient preconcentration, novel post-cell capillary HPLC

Thin-Channel Electrode Electrospray Emitter Floated 3-Electrode Cell

Counter electrode must be isolated from direct flow path



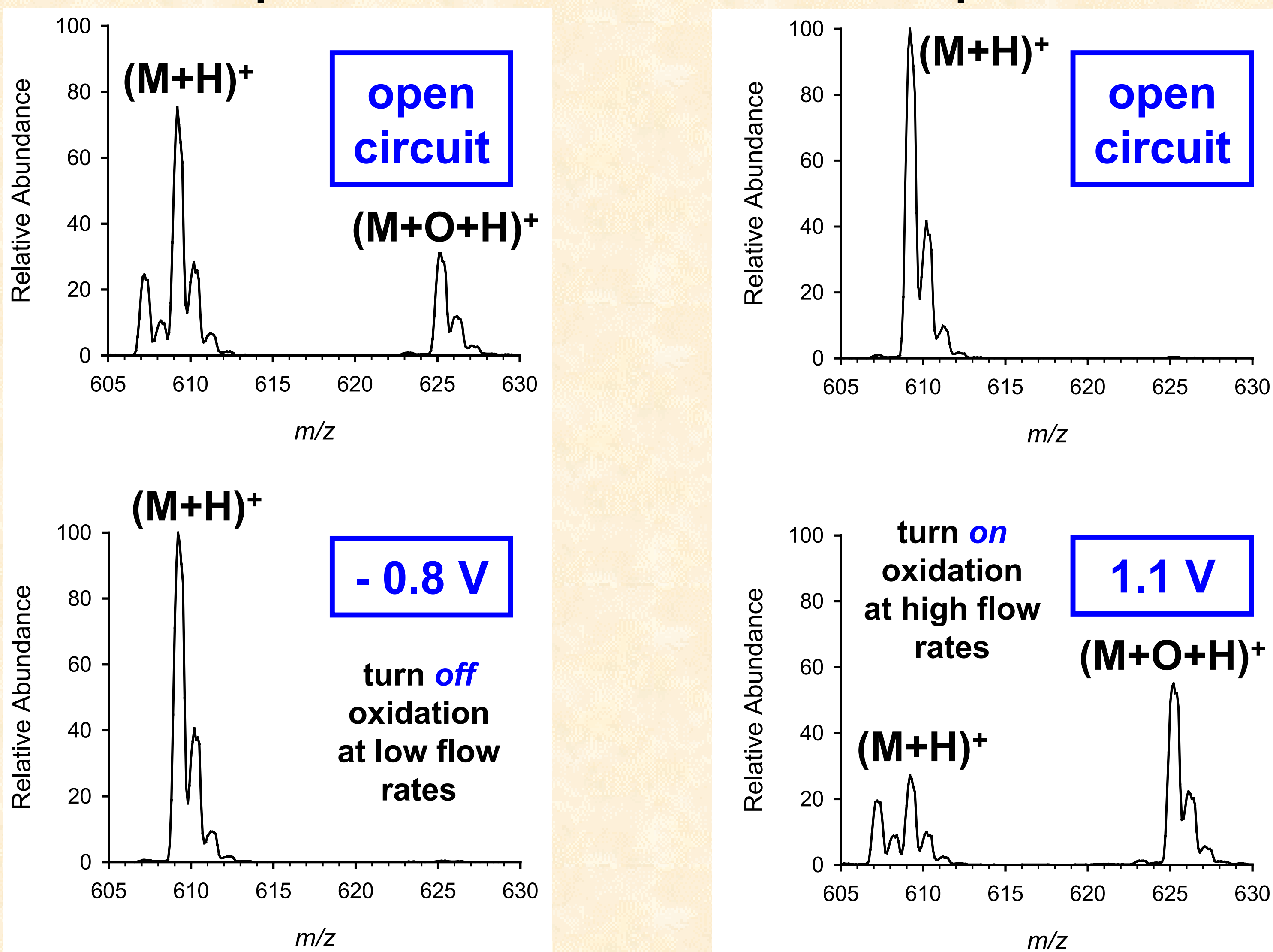


Floated 3-Electrode Cell

Control Oxidation at Low and High Flow Rate

20 µM Reserpine, 1/1 (v/v) H_2O/CH_3CN, 5 mM NH_4OAc, 0.75% HOAc

2.5 µL/min | **30 µL/min**



A Quote

"…the idea that electrochemical reactions might be taking place in an electrospray ion source was too obvious to mention. That products of such reactions are of vital significance in the overall ESI process was much less obvious. Indeed, it seems fair to say that with few exceptions the products of the electrochemical reactions have been of only minor significance in most applications of ESI-MS. However, those exceptions have turned out to be of great interest and importance."

John B. Fenn

From:

Special Feature: Discussion

"Electrochemical processes in electrospray ionization mass spectrometry"

***J. Mass Spectrom.* 2000, *35*, 939-952.**

Another Quote

"Expansion of the use of ES-MS to the analysis of analytes that have more facile electrochemistry [than the commonly analyzed peptides, proteins, and oligonucleotides], including metals, metal-ligand complexes, organometallics, conductive polymers, and many low mass organics and drugs [as well as the use of nano flow rate ES], is increasing the reports of the electrochemical nature of ES influencing ES-MS analyses."

Gary J. Van Berkel

From:

Special Feature: Discussion

"Electrochemical processes in electrospray ionization mass spectrometry"

J. Mass Spectrom. 2000, 35, 939-952.

Electrochemical Process Inherent to Electrostatic Spray Ion Sources

PEER-Reviewed Open Literature Publications