Lecture 3:
Ionization Techniques – Part II

CU- Boulder

CHEM 5181

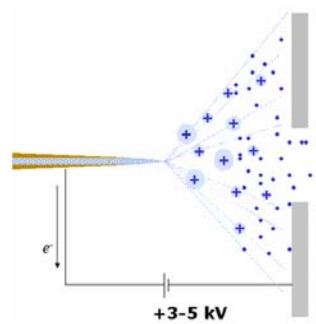
Mass Spectrometry & Chromatography

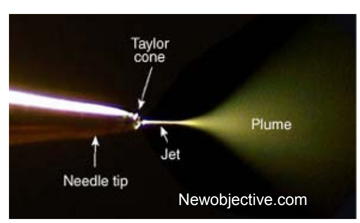
Taught by S. Kato / Slides from J. Kimmel

Fall 2009

Electrospray Ionization

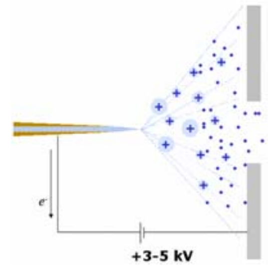
- Atmospheric pressure ionization
- Enables MS detection of **large**, non-volatile molecules (e.g., proteins) with **no fragmentation** (→Nobel Prize 2002)
  - Search "ESI-MS" = 13,000 articles
  - Fenn's 1985 A Chem paper cited 845 times
- Liquid elutes through a high voltage tip
- Coulombic explosions yield a continuous mist of bare, gas-phase ions (positive or negative)
- Conveniently coupled to liquid separations
- Characterized by multiply charged ions

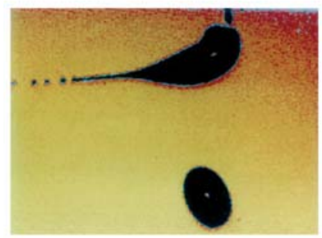




Electrospray Mechanism

- **An electrolytic analyte solution is pushed through the conductive end of capillary** (id 10-100 um) at very low flow rate (0.1-10 uL/min) held a few mm from the entrance of the MS
- **High potential** (2-4 kV) induces a strong electric field (10^6 – 10^7 Vm^{-1})
- For positive field, **cations will move towards the liquid surface** and anions will move towards the conductive tip.
- **Repulsions between adjacent cations** combined with the pull of the cations towards the grounded MS inlet cause the surface to expand into a so-called **'Taylor cone.'**





Gomez & Tang, *Phys Fluids*, 1994, 6:404–414

ESI Mech (con't)

Balance induced E field and surface tension of liquid

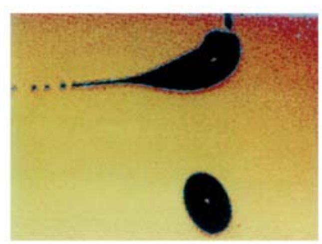
Tip of the cone elongates into a **filament**, which breaks up and emits a stream of charged droplets towards the inlet of the mass spectrometer.

Evaporation of solvent from the droplets **increases the charge density**.

At the **'Rayleigh limit,'** repulsion between cations equal surface tension, causing '**Coulombic explosions'** that produce even finer droplets.

This process of evaporation and explosion repeats until **fully desolvated ions are released**.

The release of ions occurs either by repeated fission events until total evaporation of the solvent (***Charge Residue Model***) or by direct ion emission from a charged droplet (***Ion Evaporation Model***).

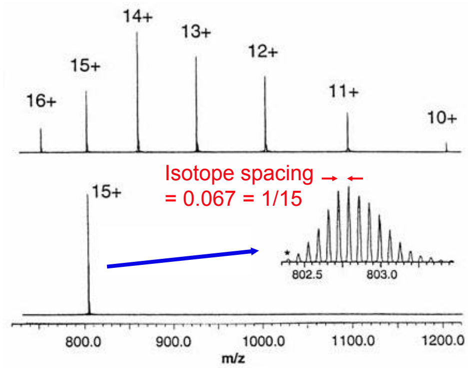


Gomez & Tang, *Phys Fluids*, 1994, 6:404–414

ESI Mass Spectrum

High charge states make *m/z* practicle for most mass analyzer types.

z can be determined by isotope distibution or sequence of peaks (see section 1.8.1 of De Hoffmann and HW #2)



ESI-MS of Cytochrome C, ~12,360 Da

From Fig 13-18 Lambert

ESI Source Design

ESI source must:

1. Move ions from solution to the gas phase
2. Transfer the gas-phase ions from atmospheric pressure to vacuum
3. Yield ion beam with maximum current and minimum kinetic energy distribution

On 1.

- Stable spray requires user optimization
- High flow rates may require nebulizing gas to form droplets

On 2.

- Heated drying gas + capillary encourage desolvation, and limits solvent analyte-adduct formation during expansion
- Pumping speed places practical limit on size of entrance aperature
- Transfer of ions between stages of decreasing pressure can result in a **total ion loss on the order of four to five orders of magnitude**

On 3.

- Harnessing expansion
- Constant Velocity = high E distribution

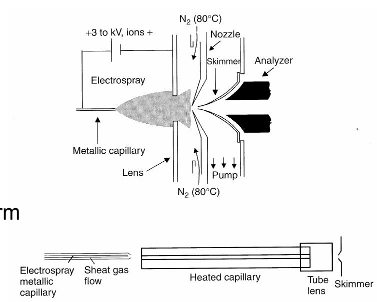
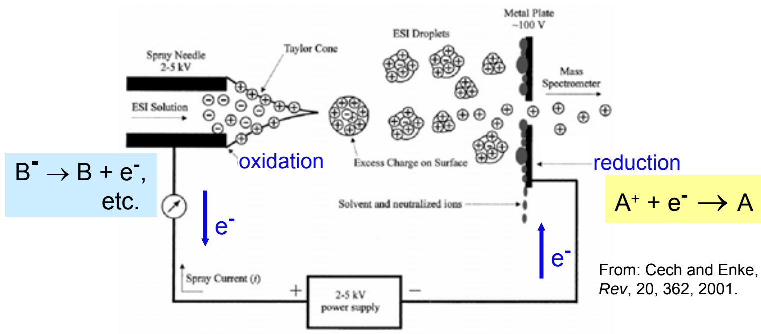


Figure 1.17
Diagram of ESI sources, using skimmers for ion focalization and a curtain of heated nitrogen gas for desolvation (*top*), or with a heated capillary for desolvation (*bottom*)

From de Hoffmann

For discussion, see: "ESI Source Design and Dynamic Range Considerations," A. P. Bruins, in "Electrospray Ionization Mass Spectrometry," R. B. Cole, 1997.

Controlled Current Electrolytic Flow Cell



From: Cech and Enke, *Mass Spec Rev*, 20, 362, 2001.

- **Electrical circuit to sustain ESI current** : (+) Terminal to tip, to counter electrode, to (-) Terminal.
- **Electrolysis** at electrodes maintains the charge balance to allow continuous production of charged droplets.
- In order to supply demanded current, potential at electrode/solution interface has value permitting the oxidation process characterized by lowest oxidation potential in solution.
- **This process determines the total # of ions that can be produced per unit time**

ESI: Concentration Dependence

- **It is the excess charge in final droplets that imparts charge to gas-phase ions**. (See Fig 1.24 in De Hoffmann)
- **ESI is sensitive to concentration**, **not flow**. Because limiting current, I_M, is dependent on oxidation process at tip.
- **ESI response can vary significantly among different analytes** that have identical concentrations
- For a system with **one analyte**, **spray current for will depend on its concentration and a analyte-specific rate constant**. $I_A = k_A[A]$
- For system of **two analytes**, A and B, $I_T = I_M = I_A + I_B$ And, currents proportional to relative desorption rates and **signal responses are coupled**. **Complicates quantification.** (See section 1.8.4 of de Hoffmann)
- **For any system, dynamic range limited at high end (~1 mM) by:**
  - Limited amount of excess charge
  - Limited space on droplet surface
  - Ion suppression
- Consider separations prior to ESI to maximize sensitivity

Nanospray

•10 – 100 **nl/min flow rate** with fine spray tip

•Flow rate and droplets 100-1000 times smaller than conventional ESI

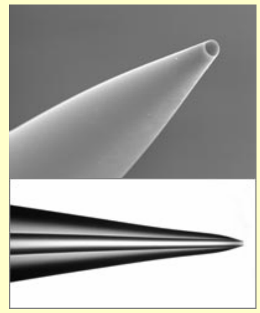
•Large proportion of analyte available for desorption from surface. **2-3 time higher ion current** than ESI at a given concentration

•Smaller tip close to orifice: narrow dispersion of droplets yields better transfer in MS

•**Orders of magnitude (2+) improvement in efficiency (analyte detected / analyte sprayed)**

•At these flow rates, ESI becomes "mass flux sensitive"

•Longer analysis times -- better SNR and/or more options in MS experiment



New Objective SilicaTips. Tip i.d. range from 5 to 30 um.

Flow rate: 20 to 1000 nL/min

See: Wilm and Mann, *A. Chem.*, 68, 1-8, 1996.

Summary

EI and CI are methods for molecular analysis of gas phase sample

APCI and ESI: molecular analysis of liquid phase

Now, **"Chemistry"** of ESI.

(S. Kato, Fall 2009)

Mass Spectrom. Rev. (2009)

ELECTROSPRAY: FROM IONS IN SOLUTION TO IONS IN THE GAS PHASE, WHAT WE KNOW NOW

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There is an advantage for users of electrospray and nanospray mass spectrometry to have an understanding of the processes involved in the conversion of the ions present in the solution to ions in the gas phase. The following processes are considered: Creation of charge droplets at the capillary tip; Electrical potentials required and possibility of gas discharges; Evolution of charged droplets, due to solvent evaporation and Coulomb explosions, to very small droplets that are the precursors of the gas phase ions; Production of gas phase ions from these droplets via the Ion Evaporation and Charge residue

Remarkably ESI can handle a vast variety of analytes such as inorganic ions as well as ionized polymers, nucleic acids, peptides and proteins that have a molecular mass from kilo- to hundreds of mega-Daltons. The analytes present in the solution may be ions, such as the inorganic metal ions M^+ and M^{2+} or negative ions such as the halide ions X^- or sulfates SO_4^{2-}. They also can be compounds that are not ionized in the solution that is sprayed. In that case the analyte is charged by association with one or more of the ions present in the solution. This charging process is part of the electrospray mechanism.

. . . .

have very low solubility for electrolytes, can be also used. For simplicity, the subsequent discussion will assume that the analyte is ionic. Only the positive ion mode will be considered.

Sorry….

Why Is Chemistry Important in ESI?

MS method	Ionizing reagent	Ion polarity & type
MALDI-TOF	solid matrix (CHCA, SA, DHB)	+/-, closed shell **(MH^+, $[M - H]^-$)**
ESI-Quadrupole-TOF (or ESI-Ion trap)	protic solvent (CH_3OH, H_2O,…) additives (HCOOH, NaCl,…)	+/-, closed shell **(MH^+, $[M - H]^-$)**
GC-Ion trap	electron	+, open shell* **(radical cation $M^{+\bullet}$)**

ESI chemistry (incl. fragmentation and CID):

Con: Many parameters to optimize

Fro: "Closed-shell chemistry", relatively easy to understand and control

*Fragmentation of radical cations will be discussed in a later lecture.

What is Known about ES Ionization of Neutrals?

(Cech and Enke, Mass Spectrom.Rev (2001); Kebarle and Verkerk, ibid (2009))

Where and how neutral species get ionized?

- in solution, in the vanishing droplets, and in the gas phase
- by adduct formation with additives: Na^+, Li^+, NH_4^+ from salts and H^+ from weak acids (ESI+); Cl^- from salts, chloroform (ESI-)
- by electrochemical reactions (e.g., H^+ from water, radical cations)
- by gas-phase proton transfer reactions ($M'H^+$ + **M** $\rightarrow$ **MH^+** + M')

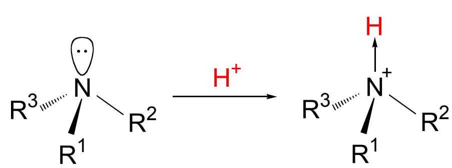
Similarity and dissimilarity between gas-phase and solution-phase ionization chemistries?

- Proton transfers in solution (by pKa) and in the gas phase (by proton affinity) do not necessarily parallel. ⇔ **Some parallels**

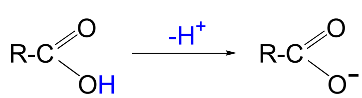
So many unknowns, but gas-phase ion chemistry is a good starting point (and is quite useful) for getting ideas.

ESI Chemistry: Rule of Thumb

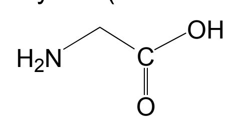
The ESI+ usually detects O, N, and S-containing species, in addition to some specific hydrocarbons like isoprene, terpenes, and aromatics, as protonated neutral MH^+.



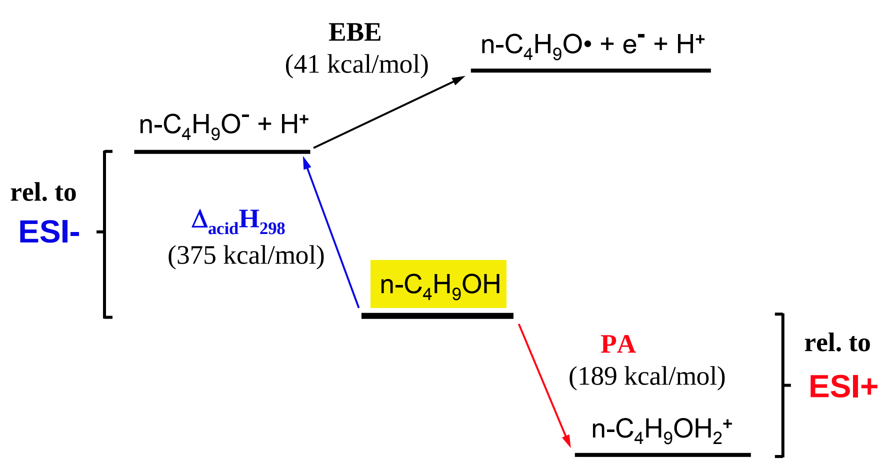
The ESI- usually detects acids (e.g, carboxylic acids RCOOH and inorganic acids) and hydrosulfides (RSH), as deprotonated neutral $[M - H]^-$.



Glycine (bifunctional)



Gas-Phase Thermochemical Scheme



Gas-Phase Thermochemistry (kcal/mol)

(All data from NIST Chemistry Webbook)

neutral (M)	$\Delta_{acid}H_{298}$	*EBE ($[M-H]^-$)	H^+ affinity (PA)	**PA - PA (H_2O)	Li^+ affinity	Na^+ affinity
C_2H_6	420.1	***-0.260	142.5	-22.5	n/a	n/a
CH_4	416.7	0.080	129.9	-35.1	n/a	7.2
NH_3	403.4	0.771	204.0	39.0	39.1	24.4
C_6H_6	401.7	1.096	179.3	14.3	38.5	22.8
H_2O	390.3	1.828	165.0	0.0	32.3	24.0
CH_3OH	382.0	1.572	180.3	15.3	36.8	23.2
i-PrOH	375.1	1.874	189.5	24.5	41.3	27.0
t-BuOH	374.7	1.780	191.8	26.8	42.5	28.0
CH_3CN	372.9	1.543	186.2	21.2	43.0	30.5
$(CH_3)_2CO$	368.8	1.758	194.0	29.0	44.5	31.2
CH_3CHO	365.8	1.825	183.7	18.7	41.3	27.1
HCHO	394.5	0.313	170.4	5.4	36.0	n/a
CH_3SH	357.6	1.867	184.8	19.8	n/a	n/a
t-BuSH	352.5	2.070	195.1	30.1	n/a	n/a
CH_3COOH	348.1	3.470	187.3	22.3	41.5	n/a
HCOOH	346.2	****3.510	177.3	12.3	n/a	n/a
PhCOOH	340.1	3.750	196.2	31.2	n/a	n/a
Glycine	341.6	3.690	211.9	46.9	n/a	38.5

*Electron binding energy in eV for deprotonated $[M - H]^-$ anion

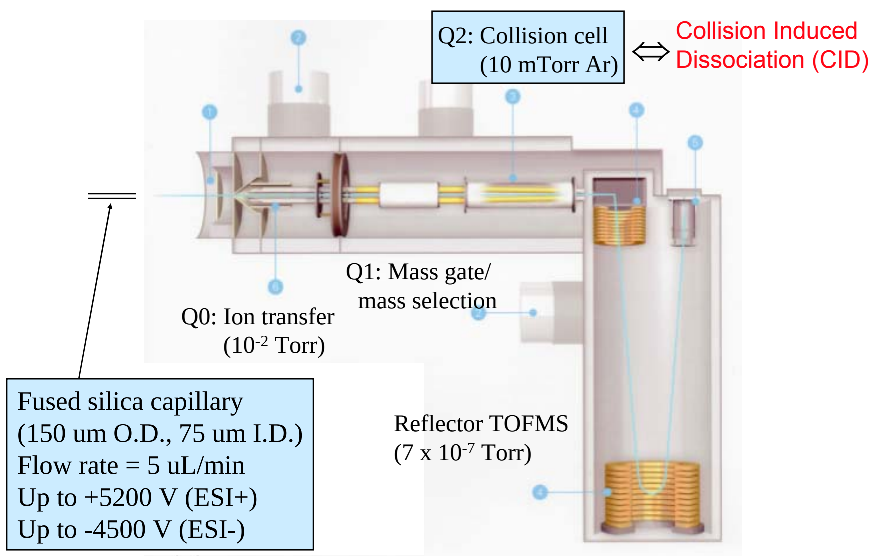
**Enthalpy for proton transfer: $M + H_3O^+ \longrightarrow MH^+ + H_2O$

ESI Chemistry in Practice

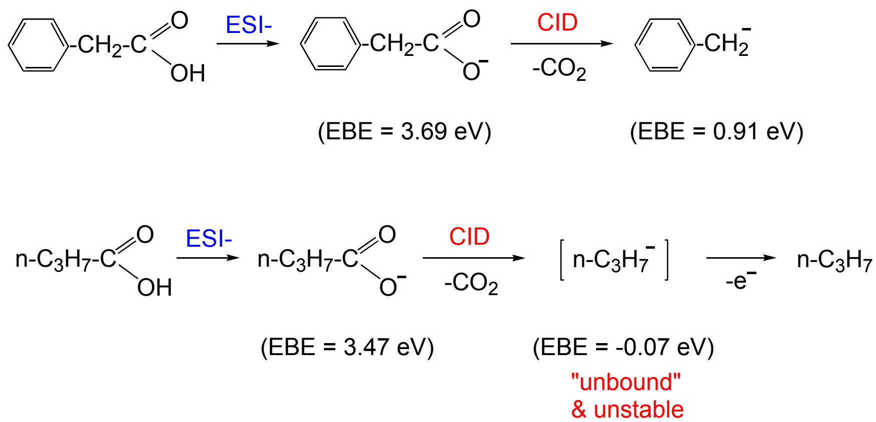
Analyte	ESI polarity	ESI reagent	ΔPA (vs. H_2O, $R=CH_3$)
carboxylic acid O ‖ R-C-OH	- (or +)	methanol (or ACN + trace H_2O)	22.3 kcal/mol
ketones O ‖ R-C-CH_3	+	methanol (or ACN + trace H_2O)	29.0 kcal/mol
aldehydes O ‖ R-C-H	+	methanol (or ACN + H_2O) + HCOOH, LiCl, or NaCl	18.7 kcal/mol

cf. Lithium ion attachment MS (Selvin, Iwase and Fujii, Anal.Chem.**74**, (2002) 2053

ESI-Quad-TOF MS instrument (ABI Pulsar)

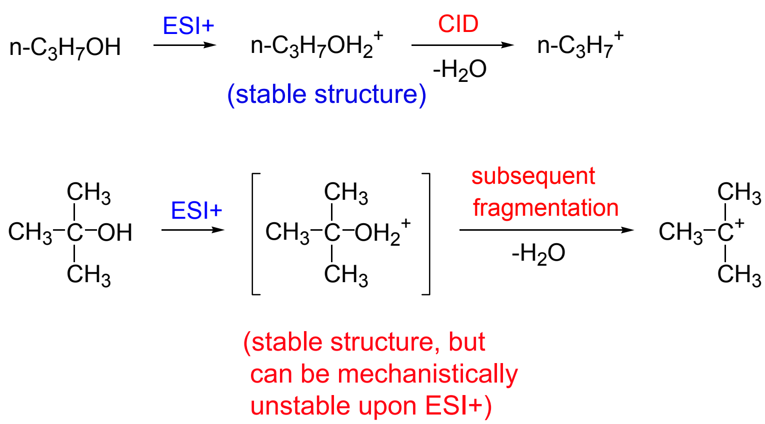


ESI- Chemistry: Stability of Formed Ions



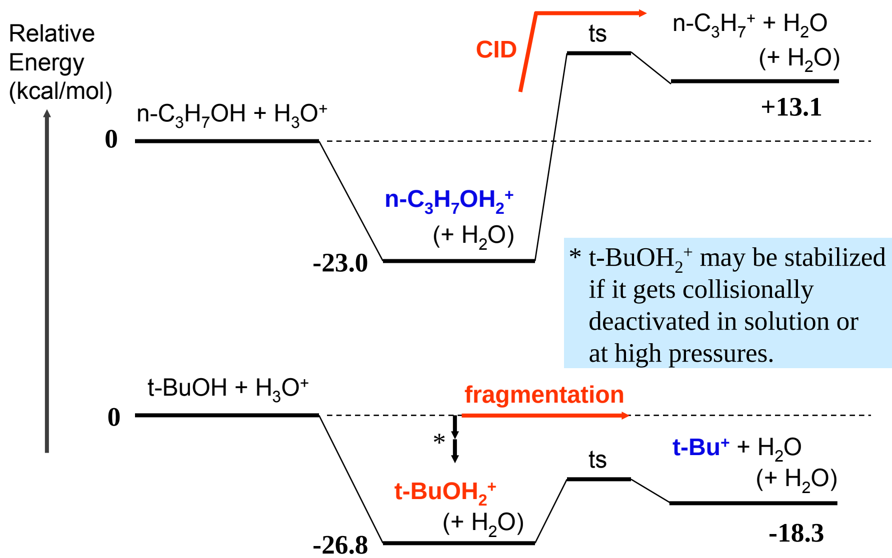
Decarboxylation is a characteristic mode in collision-induced dissociation (CID) of carboxylates.

ESI+ Chemistry: Stability of Formed Ions



For difficult analytes, lithiation or sodiation may be used to detect the intact molecular ions.

ESI+ Chemistry: Stability of Formed Ions (con't)



Summary: ESI Chemistry

ESI chemistry has many control parameters, but with adequate knowledge of organic chemistry and expertise, ESI-MS is one of the quickest and most efficient techniques in mass spectrometry.