Electrospray and Ambient Ionization Sources

CU- Boulder
CHEM 5181
Mass Spectrometry & Chromatography
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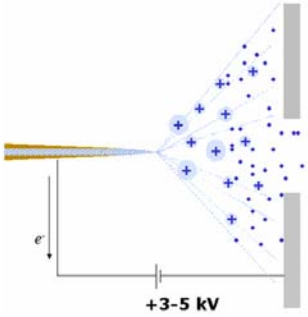
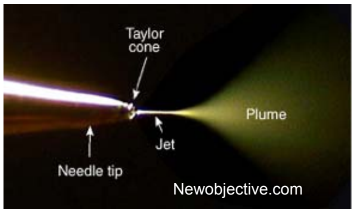
With some slides from Drs. Joel Kimmel and Shuji Kato
Last update: Oct. 2014

Electrospray Ionization

- *At atmospheric pressure*
- 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





Newobjective.com

Electrospray Mechanism

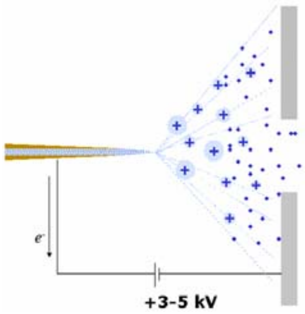
•**An electrolytic analyte solution is pushed through the conductive end of capillary** (id 10-100 µm) at very low flow rate (0.1-10 µL/min) held a few mm from the entrance of the MS

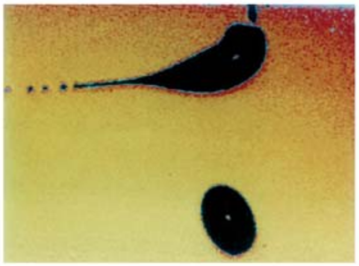
•**High potential** (2-4 kV) induces a strong electric field ($10^6 - 10^7$ Vm^{-1})

•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.'**

Video1: http://www.youtube.com/watch?v=K-GhE1uWoV4

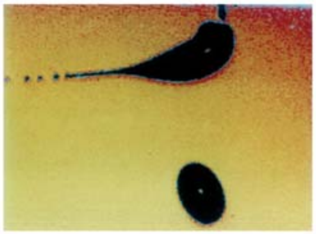




Gomez & Tang, *Phys Fluids*, 1994, 6:404–414

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

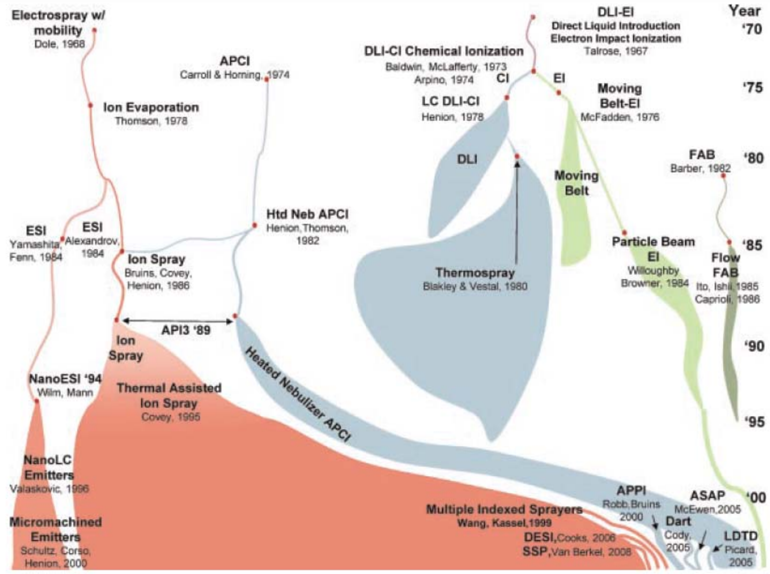


FIGURE 1. A history of the development of LC/MS instrumentation as viewed in histomap format. The "y" axis is year and the "x" axis is the relative adoption of the technique at that point in time estimated by the extent of commercial adaptation as well as publications and activity in academic research institutions. The concept of a histomap was borrowed from a Rand McNally publication (Sparks, 1952). The year 1989 marks the time of the first commercial API ESI mass spectrometer (Sciex API 3). Names and dates refer to specific citations in the Reference section.

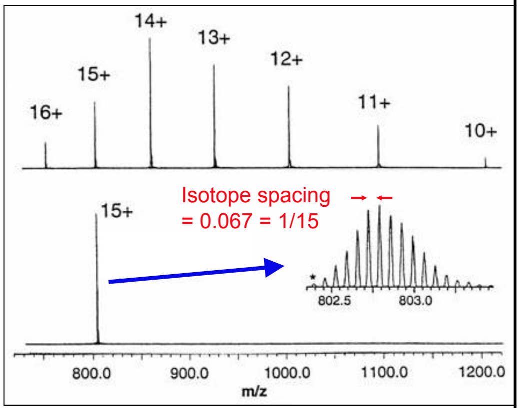
Covey et al., Atmospheric Pressure Ion Sources, Mass Spec Rev 2009

ESI Mass Spectrum

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

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

From Fig 13-18 Lambert



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

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

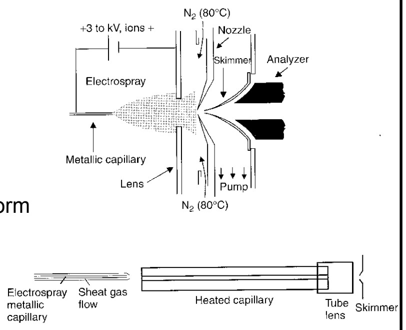


Figure 1.17
Diagram of ESI sources, using skimmers for ion focalization and a curtain of heated nitrogen gas for desolvation (*top*), or with a heated capillary for desolvation (*bottom*)

From de Hoffmann

On 1.

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

On 2.

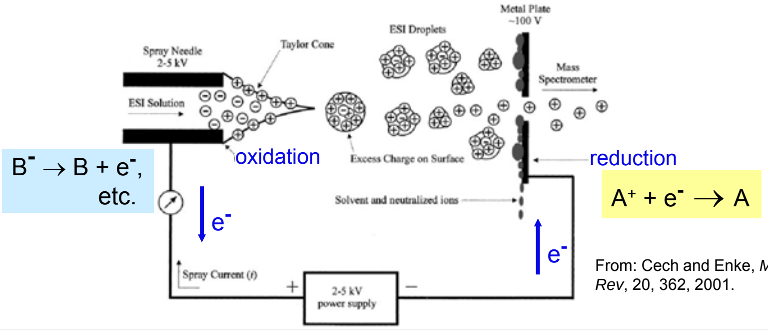
- Heated drying gas + capillary encourage desolvation, and limits solvent analyte-adduct formation during expansion
- Pumping speed places practical limit on size of entrance aperture
- Transfer of ions between stages of decreasing pressure can result in a **total ion loss on the order of four to five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

On 3.

- Harnessing expansion
- Constant Velocity = high E distribution

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



$B^- \rightarrow B + e^-$, etc.

$A^+ + e^- \rightarrow A$

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

•**Electrical circuit to sustain ESI current** : (+) Terminal to tip, to counter electrode, to (-) Terminal.

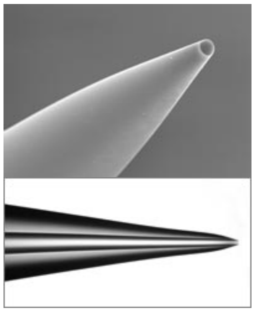
•**Electrolysis** at electrodes maintains the charge balance to allow continuous production of charged droplets.

•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

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 µm.

Flow rate: 20 to 1000 nL/min

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

Kebarle MSR 2009

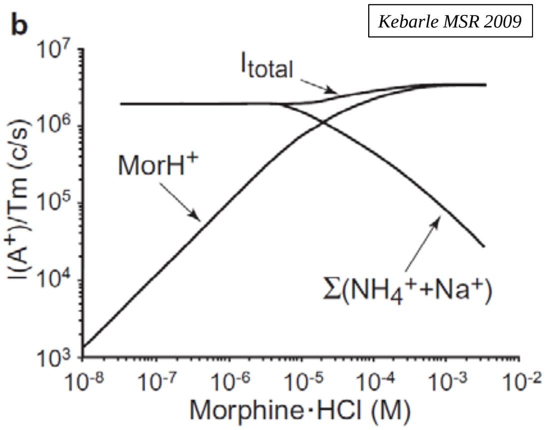
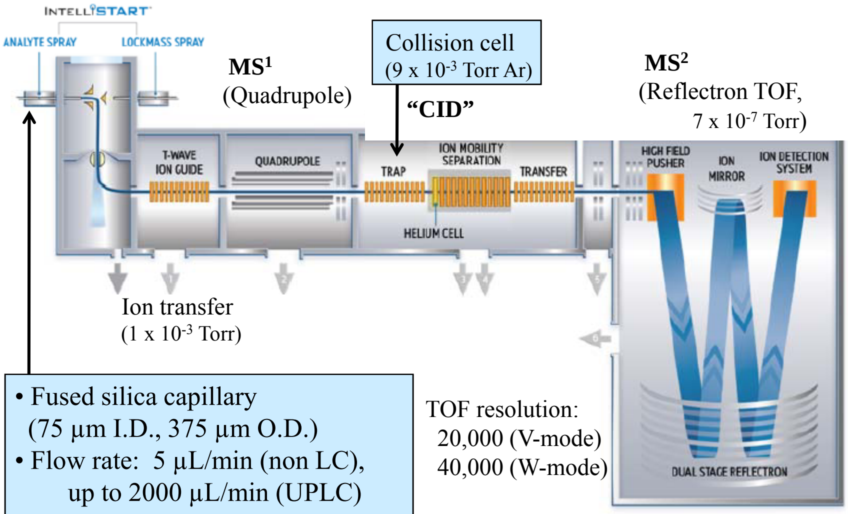


FIGURE 7. **a**: Electrospray current (amp) with increasing concentration of analyte MorphineHCl. Due to presence of impurity ions (Na^+ and NH_4^+) at a 10^{-5} M level. I_{total} remains constant up to the point where the analyte $MorH^+$ reaches concentrations above 10^{-5} M. **b**: Analyte $MorH^+$ ion intensity (corrected for mass dependent ion transmission, Tm, of quadrupole mass spectrometer used) is proportional to concentration of MorphineHCL up to the point where the MorphineHCl concentration approaches concentration of impurity ions. (Reprinted from Kebarle & Tang, 1993, with permission from the American Chemical Society, copyright 1993.)

Ion Supression

- One analyte can suppress the signal of others
- ESI practitioner: “NMR is quantitative but not very sensitive, and MS is very sensitive but not quantitative”
  - *Shocking to me!*

Synapt G2 ESI-Quad-TOF MS (Waters)



MS^1
(Quadrupole)

Collision cell
(9×10^{-3} Torr Ar)

"CID"

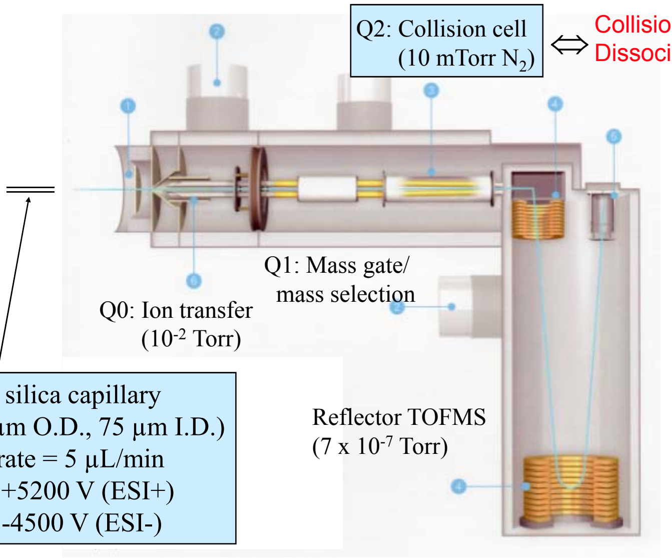
MS^2
(Reflectron TOF,
7×10^{-7} Torr)

Ion transfer
(1×10^{-3} Torr)

- Fused silica capillary
  (75 µm I.D., 375 µm O.D.)
- Flow rate: 5 µL/min (non LC),
  up to 2000 µL/min (UPLC)
- +3000 V (ESI+), −2000 V (ESI−)

TOF resolution:
20,000 (V-mode)
40,000 (W-mode)

ESI-Quad-TOF MS instrument (ABI Pulsar)



Q2: Collision cell
(10 mTorr N_2) ⇔ Collision Induced Dissociation (CID)

Q1: Mass gate/
mass selection

Q0: Ion transfer
(10^{-2} Torr)

Fused silica capillary
(150 µm O.D., 75 µm I.D.)
Flow rate = 5 µL/min
Up to +5200 V (ESI+)
Up to -4500 V (ESI-)

Reflector TOFMS
(7×10^{-7} Torr)

Atmospheric Pressure Desorption Ionization

2000: AP-MALDI is first atmospheric pressure ionization technique for condensed-phase analyte. Burlingame *et al*, *A Chem*, 652, 2000

2004: Desorption Electrospray Ionization (DESI) Cooks *et al*, *Science*, 2004, 471-473

2005: Direct Analysis in Real Time (DART) Cody *et al, A. Chem*, 2005, 2297.

2005 – Present: Rapid development, application, and evolution of these methods (e.g., MS conferences may have multiple sessions dedicated to DESI and DART)

*** These methods would make for interesting journal presentation*

Desorption Electrospray Ionization

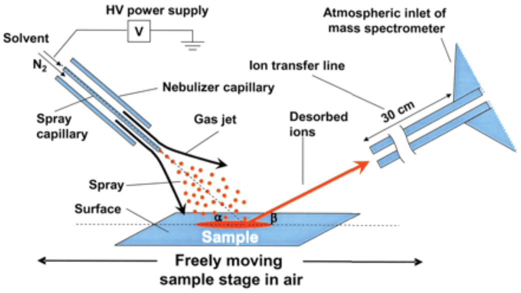


Image from: Cooks *et al*, *Science*, 2004, 471-473

Photos from: http://www.prosolia.com/index.html

Advance: (DESI and DART) **Analysis in free ambient environment**, sample potentially subject to arbitrarily chosen processes during MS analysis.

- Applicable to small molecule organics and large biomolecules in solids, liquids, and adsorbed gases.
  - Natural products in plant material
  - High throughput of pharmaceuticals
  - Biological tissue and fluids
  - Forensics / Public safety
- Typically **no pre-treatment**. Sample is sprayed with an electrically charged aqueous mist. Released ions are transported through air to MS interface (which may be long transfer line).

Desorption Electrospray Ionization

- Solution can be tuned to make process **selective**.
- Relative movement of sample and spray enables spatial resolution down to 50 um, for **imaging of surfaces**
- The momentum-transfer collisions of DI in vacuum are not applicable, as **projectiles have low kinetic E**.

Two mechanisms:

ESI-like: DESI produces multiply charged ions from biological macromolecules. Ionization believed to occur through formation of charged droplets, after liquid spreads across surface.

APCI-like: For molecules that cannot be analyzed by ESI (e.g., non-polar compounds) see singly charged species, indicative of charge transfer between liquid and surface or ion-molecule reactions. Requires potential (2kV) between sprayer and surface.

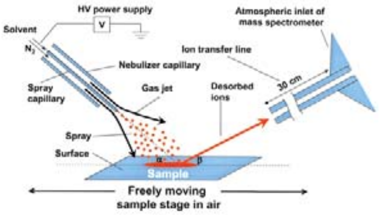
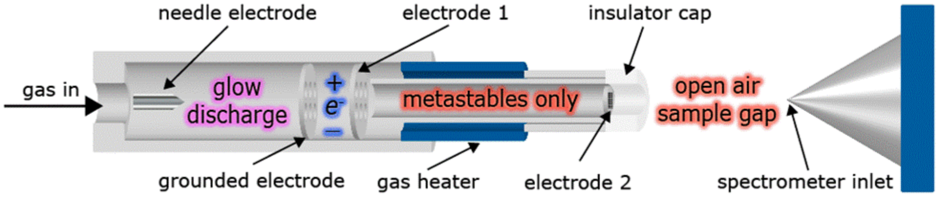


Image from: Cooks *et al*, *Science*, 2004, 471-473

DART Ion Source



From Wikipedia

“Chip” Cody

- Glow discharge makes ions and e- (removed by field)
- Glow discharge also makes metastable atoms of e.g. He

$$He(2^3S) + H_2O \rightarrow H_2O^{+\bullet} + He(1^1S) + e^-$$

- He metastables ionize H_2O

$$H_2O^{+\bullet} + H_2O \rightarrow H_3O^+ + OH^\bullet$$

- Protonated water clusters with H_2O

$$H_3O^+ + nH_2O \rightarrow [(H_2O)_n H]^+$$

- Water clusters ionize analyte “S”

$$[(H_2O)_n H]^+ + S \rightarrow SH^+ + nH_2O$$

http://en.wikipedia.org/wiki/DART_ion_source

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

Summary

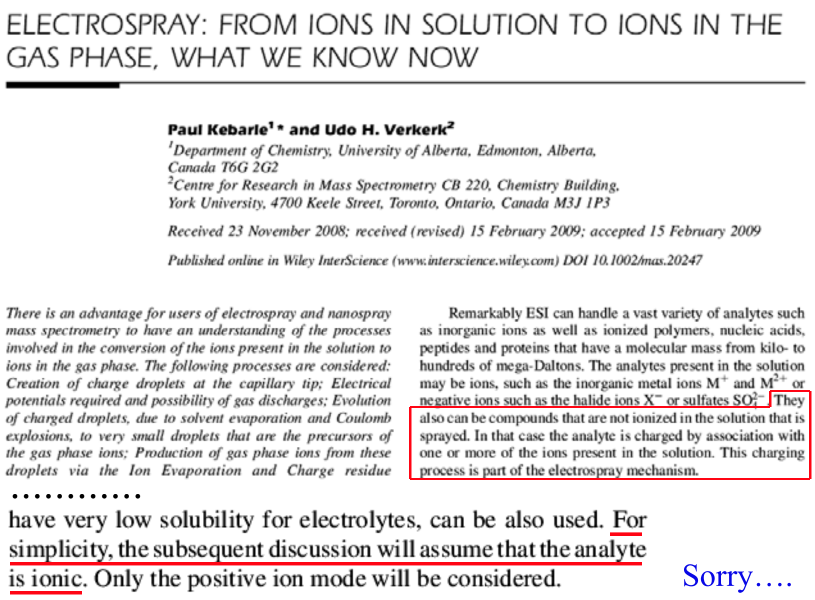
EI and CI are methods for molecular analysis of gas phase sample

APCI and ESI: molecular analysis of liquid phase

Now, **“Chemistry”** of ESI.

(S. Kato, Fall 2011)

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** → **MH^+** + M')

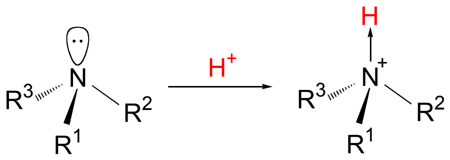
Similarity and dissimilarity between gas-phase and solution-phase ionization chemistries?

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

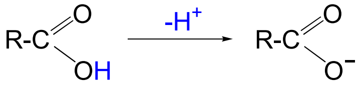
So many unknowns, but gas-phase ion chemistry is a good starting point (and is quite useful) for getting ideas.

ESI Chemistry: Rule of Thumb

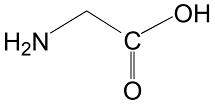
The ESI+ usually detects O, N, and S-containing species, in addition to some specific hydrocarbons like isoprene, terpenes, and aromatics, as protonated neutral MH^+.



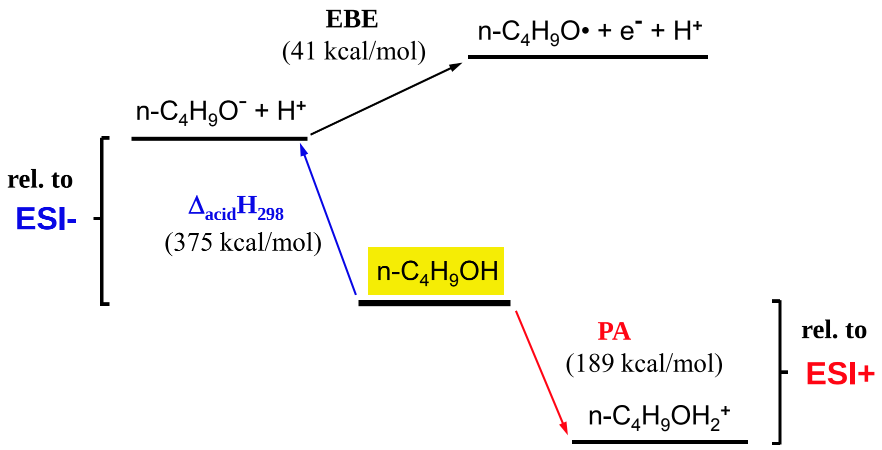
The ESI- usually detects acids (e.g, carboxylic acids RCOOH and inorganic acids) and hydrosulfides (RSH), as deprotonated neutral $[M - H]^-$.



Glycine (bifunctional)



Gas-Phase Thermochemical Scheme



EBE
(41 kcal/mol)

n-$C_4H_9O\bullet + e^- + H^+$

n-$C_4H_9O^- + H^+$

rel. to
ESI-

$\Delta_{acid}H_{298}$
(375 kcal/mol)

n-C_4H_9OH

PA
(189 kcal/mol)

n-$C_4H_9OH_2^+$

rel. to
ESI+

Gas-Phase Thermochemistry (kcal/mol)

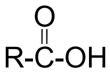
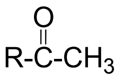
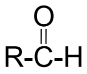
(All data from NIST Chemistry Webbook)

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

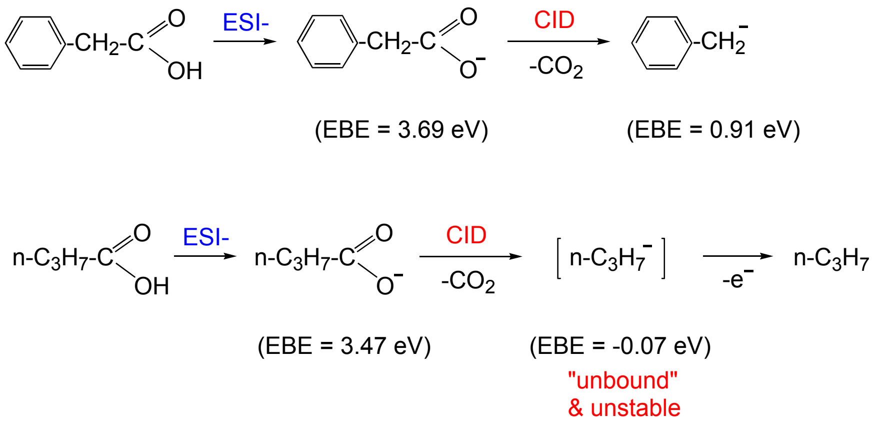
**Enthalpy for proton transfer: $M + H_3O^+ \longrightarrow MH^+ + H_2O$

ESI Chemistry in Practice

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

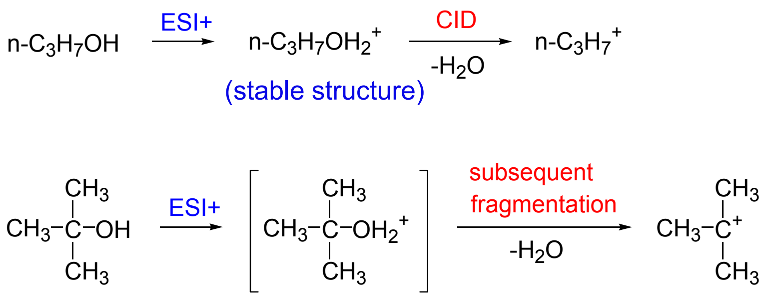
cf. Lithium ion attachment MS (Selvin, Iwase and Fujii, Anal.Chem.**74**, (2002) 2053

ESI- Chemistry: Stability of Formed Ions



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

ESI+ Chemistry: Stability of Formed Ions



$$n\text{-}C_3H_7OH \xrightarrow{\text{ESI+}} n\text{-}C_3H_7OH_2^+ \xrightarrow[-H_2O]{\text{CID}} n\text{-}C_3H_7^+$$

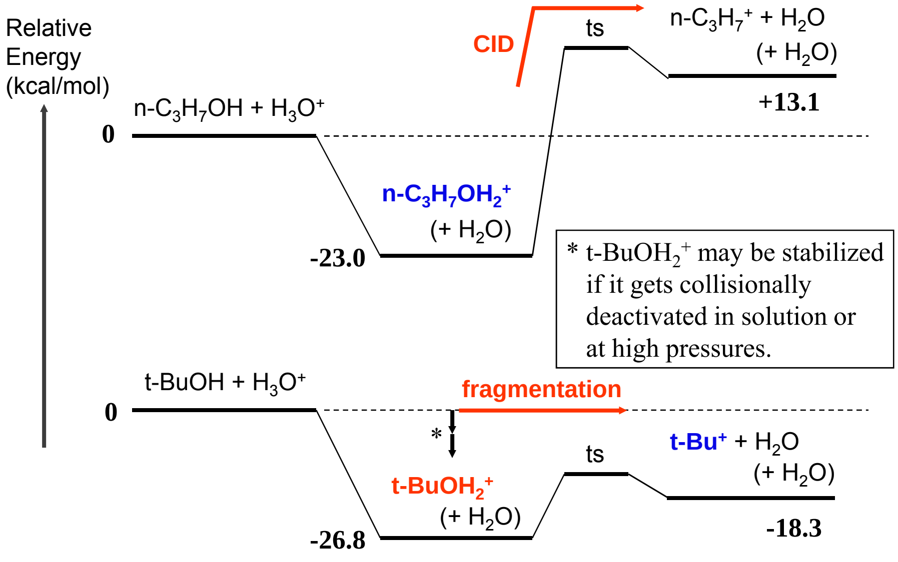
(stable structure)

$$CH_3\text{-}C(CH_3)_2\text{-}OH \xrightarrow{\text{ESI+}} \left[CH_3\text{-}C(CH_3)_2\text{-}OH_2^+\right] \xrightarrow[-H_2O]{\text{subsequent fragmentation}} CH_3\text{-}C^+(CH_3)_2$$

(stable structure, but can be mechanistically unstable upon ESI+)

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

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



* t-BuOH$_2^+$ may be stabilized if it gets collisionally deactivated in solution or at high pressures.

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.