“Real”
(Modern)
Mass Spectrometry

(Part I)

Alex J. Roche
MS is fundamentally important

• If you want to publish a **new compound**, 3 pieces of data are required:
  – relevant NMR (H, C, F, P, Pt, N, D, B, …)
  – **Mass Spectrometry** (low resolution)
  – Elemental Analysis (CHN) or **HRMS**

• Confirmation of a compound usually requires the 1\textsuperscript{st} two

• Industrially, GCMS / HPLCMS is the most common general Chemical Identification / Characterization tool

• ACS accreditation guidelines state “**instruments and equipment in a good undergraduate chemistry program include** …gas chromatography mass spectrometry (GC-MS), and apparatus or instruments for Mass Spectrometry”.
Mass Spectrometry

“What do you know about that?”

• Basic concept? *(what is it?)*

• What’s the point? *(what information does it provide?)*

• Underlying physics? *(why does it work?)*

• Underlying mechanics? *(how does it work?)*
What we (I) teach

Ions are generated, bent by magnetic field, and separated according to m/z ratio.

→ molecular weight information, and structural information
Agilent Quote

(After significant bartering)
$228,000 = Agilent Technologies Triple Quad HPLCMS with Multimode Source Instrument.

<table>
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<tr>
<th>Product/Description</th>
<th>Qty/Unit</th>
<th>Unit List Price</th>
<th>Discount Amount</th>
<th>Extended Net Price</th>
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<td>G6410AA LCMS Triple Quad ES Bundle</td>
<td>1.000 EA</td>
<td>204,999.00 USD</td>
<td>49,199.76-</td>
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<td>MS Triple Quad ES Bundle, Includes G6410A 3Q, API-ES source, Mass Hunter Quant, Qual and Reporting SW, PC and Printer, 24 hrs I&amp;F Image Recovery Service included.</td>
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<tr>
<td>G1978A Multimode ESI/APCI Source</td>
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<td>21,900.00 USD</td>
<td>5,102.71-</td>
<td>16,797.29</td>
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<td>Includes API-ES/APCI spray chamber, Nebulizer and test mix kit. Requires 32-bit ChemStation Software.</td>
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MOST COMMON MODERN STANDARD MS INSTRUMENT
• Where is the triple quad?
• Where is the ESI or APCI?
• “...This type of ionization is compatible with triple quadrupole and ion-trap analyzers. Not so much with magnetic sector instruments (if you can still find one)...”
Mass Spec Trivia

• 1803 Dalton “Mass consists of atoms…”
• 1911 Thompson 1st mass spectrometer
• 1940 Berry Electron Impact Ionization for organics
• 1980 ESI, FAB, MALDI
• Up to current day, MS fundamental characterization tool.
• 2002 Nobel Prize MALDI / ESI (Tanaka / Fenn)
• (4th MS area Nobel Prize)

• Mass is measured in a.m.u. = Daltons, Da.
  ($^{12}\text{C} = 12 \text{amu} = 12 \text{Da}$)
• m/z is measured in Thompsons
Variables for MS

- **Sample Type**
  
  *(Solid, liquid, gas, ionic, pure or mixture, in solution, molecule, polymer, protein, ...)*

- **Sample Introduction**
  
  *(Direct insertion, matrix, GC, HPLC, ...)*

- **Ionization Technique** *(this week)*

- **Type of Probe (Mass analyzer)** *(next week)*
Ionization Techniques

- Glow discharge (GD)
- Electron impact ionization (EI)
- Chemical ionization (CI)
- Field ionization (FI)
- Inductively coupled plasma (ICP)
- Fast atom bombardment (FAB)
- Secondary ion mass spectrometry (SIMS)
- Thermospray (TSI)
- Ionspray (IS)
- Electrospray (ESI)
- Plasma Desorption (PD)
- Laser Desorption (LD)
- Matrix-assisted laser desorption/ionization (MALDI)
Mass Analyzers

- Magnetic sectors
- Quadrupole (Q)
- Triple Quad (QQQ, QqQ or 3Q)
- Ion cyclotron with Fourier transformation (ICR-FT-MS)
- Ion trap (IT), linear trap (LT)
- Time-of-flight mass spectrometer (TOF)
- Hybrids (TOF-TOF, QTOF, etc)
Ionization Techniques

EI
CI
MALDI

ESI
APCI (and mention APPI)
Ion Source

• How (and where) do we get ions?

• Before the vacuum – **Atmospheric Pressure Ionization**
  (in solution e.g. output from an HPLC)

• Or if your analyte is “pure” / from a GC (inside the vac)
(a) Electron Ionization

- If “pure” compound (by either direct insertion or GC/MS)
- We can use electrons to knock out other electrons.
- \[ M + e^- \rightarrow M^+ + 2e^- \]
Electron Ionization

•http://www.shsu.edu/~chm_tgc/sounds/flashfiles/GC-MS.swf
Effect of Ionization Energy

Ionization Energy Dependence, using Benzoic Acid $\text{C}_7\text{H}_6\text{O}_2 = 122$

Higher Energy leads to more fragmentation
Molecular Ion or Fragmentation?

Isomers can be differentiated by their fragmentation

\[ C_6H_{12}O = 100 \]
(b) Chemical Ionization

- A reactant gas is ionized (by EI) and then collided with our analyte, and this process ionizes our analyte (usually via protonation)

- **Methane:**
  - \( \text{CH}_4 + e \rightarrow \text{CH}_4^+ + 2e \rightarrow \text{CH}_3^+ + \text{H}^+ \)
  - \( \text{CH}_4^+ + \text{CH}_4 \rightarrow \text{CH}_5^+ + \text{CH}_3^* \)
  - \( \text{CH}_4^+ + \text{CH}_4 \rightarrow \text{C}_2\text{H}_5^+ + \text{H}_2 + \text{H}^* \)

- **Isobutane:**
  - \( i\text{-C}_4\text{H}_{10} + e \rightarrow i\text{-C}_4\text{H}_{10}^+ + 2e \)
  - \( i\text{-C}_4\text{H}_{10}^+ + i\text{-C}_4\text{H}_{10} \rightarrow i\text{-C}_4\text{H}_9^+ + \text{C}_4\text{H}_9 + \text{H}_2 \)

- **Ammonia:**
  - \( \text{NH}_3 + e \rightarrow \text{NH}_3^+ + 2e \)
  - \( \text{NH}_3^+ + \text{NH}_3 \rightarrow \text{NH}_4^+ + \text{NH}_2^* \)
  - \( \text{NH}_4^+ + \text{NH}_3 \rightarrow \text{N}_2\text{H}_7^+ \)

(Shown for Cl+)

Can use the same source and instrument for EI and CI, just need additional inlet for reactant gas.
EI or Cl?

Cl generates more molecular ions, or precisely [M+H]+ ions.

C₅H₉NO₂ = 115

These techniques are COMPLEMENTARY

ALL GCMS IS EI or CI
(c) MALDI

- Matrix Assisted Laser Desorption Ionization

1. Sample is mixed with **matrix** (X) and dried on plate.

2. Laser flash ionizes matrix molecules.

3. Sample molecules (M) are ionized by proton transfer:
   \[ XH^+ + M \rightarrow MH^+ + X. \]

- Solid Phase samples
- Usually for peptides / proteins / small synthetic polymers, or other large ‘molecules’
Common Matrices

Desirable matrices have:
- high light absorption coefficient at laser wavelength (337nm for N₂ laser)
- miscibility with analyte
MALDI

- Soft ionization yielding [M-H]^+
- Usually gives simple spectra with z = 1
- Complementary to ESI
- MALDI and ESI = MS of Biopolymers

Disadv.
- Low mass analytes ‘lost’ in matrix background
- Laser focusing on the target problems
Problems with Previous Methods

- If compounds are:
  - not volatile
  - in solution
  - a mixture...
  ...then there are some problems with the previous ionization methods.

- Nowadays, almost all modern MS machines have an HPLC input, and have Atmospheric Pressure Ionization (API) interfaces.
Atmospheric Pressure Ionization

• Most common types:
  
  - Electrospray Ionization (ESI)
  - Atmospheric Pressure Chemical Ionization (APCI)

• Both are ‘soft’ ionizations occurring at atmospheric pressure.

• ESI is considered the most general, widely applicable ionization for small and large molecules at femtomolar sensitivities (10⁻¹⁵ M).
(d) ESI

• ESI allows production of molecular ions directly from samples in solution.

• HPLC outlet connected to high voltage capillary / needle surrounded by N₂ flow.

• Effluent is charged and nebulized
  \( \rightarrow \) charged droplets of solvent and analyte.

• Droplets are charged by voltage, solvent evaporates, droplet size decreases, until it explodes from repulsion.
ESI contd.
(e) APCI

• Atmospheric Pressure Chemical Ionization:

• The effluent exits a heated but UNCHARGED capillary.
• A corona discharge needle at a several kilovolt potential is close by and this ionizes the solvent, which in turn ionizes the analyte.

• Corona effect (plasma) is partial discharge around a conductor placed at a high potential, which leads to ionization and electrical breakdown of the proximate atmosphere.

• (Natural corona = St. Elmo’s Fire)
APCI

Solvent / atmosphere molecules get protonated by corona

\[ S \rightarrow SH^+ \]

which then ionize the analyte

\[ M \rightarrow [MH]^+ \]

*(Atmospheric pressure Photo Ionization (APPI) is a new method using UV lamp and photons instead of the corona).*
ESI or APCI?

- Cpds generally respond better to one of ESI or APCI.
- Complementary techniques and information.

ESI more polar cpds & higher mass

APCI more nonpolar & lower mass
Why Choose?

- ESI and APCI (almost) cover all possible samples.
- Normally have to switch components to go from one ionization mode to the other.
- Be very nice (convenient / quicker / safer) to do both ESI and APCI on the same sample run.
- MultiMode source (MM) does this!
- HPLC effluent, ‘half’ gets ESI, the other ‘half’ gets APCI, both go into the MS.

(....Agilent Video of Multimode)
• Multimode Video from Agilent
Next week, we’ll address the mass analyzer, and molecular weights.