Mass spectrometry

Dr. Kevin R. Tucker

What is mass spectrometry?

- A versatile analytical technique used to determine the composition of chemical samples either at the atomic or molecular level.
- Ionic forms of samples are separated according to differences in component *mass-to-charge (m/z) ratio*.
  - Signals from each different isotopic composition
  - Isotopes usually stable
  - Keep in mind charge, z, in (+1, +2, +3, etc.); not knowing z can result in mass errors by a factor of 2, 3, etc.

Dichloromethane CH_2Cl_2

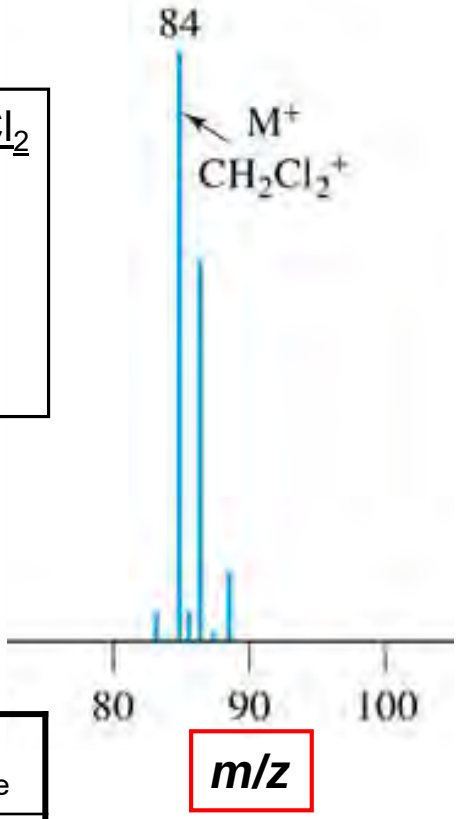
$^{12}C^{1}H_2{}^{35}Cl_2 = 84$

$^{13}C^{1}H_2{}^{35}Cl_2 = 85$

$^{12}C^{1}H_2{}^{35}Cl^{37}Cl = 86$

$^{13}C^{1}H_2{}^{35}Cl^{37}Cl = 87$

$^{12}C^{1}H_2{}^{37}Cl_2 = 88$



Isotope	% Natural Abundance
^{12}C	98.93
^{13}C	1.07
^{35}Cl	75.77
^{37}Cl	24.23

Textbook value is a weighted average
Cl At. wt. = 35.453

Basic instrumental setup for mass spectrometry

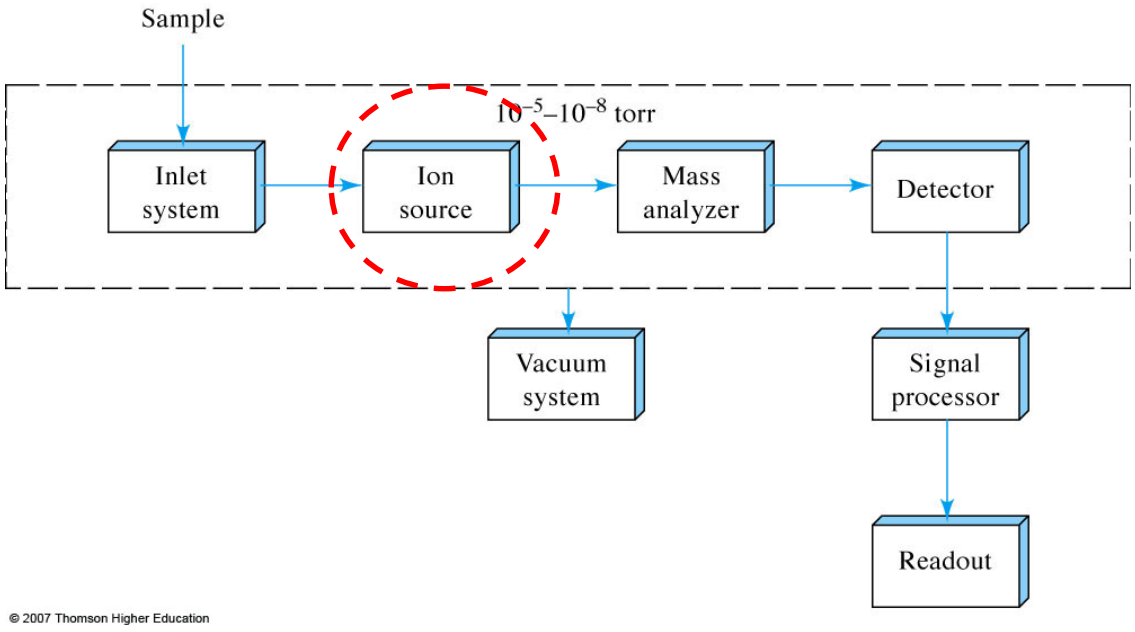
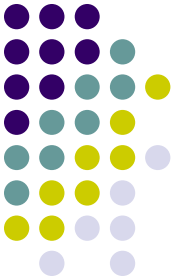


Fig. 20-11

Molecular mass spectrometry
Chapter 20

Generating ions for molecular mass spectrometry



- Two major classes
  - **Gas phase**: Sample vaporized then ionized
  - **Desorption**: Solid or liquid sample is directly converted into gas phase ions

TABLE 20-1 Ion Sources for Molecular Mass Spectrometry

Basic Type	Name and Acronym	Ionizing Agent
Gas phase	* Electron impact (EI)	Energetic electrons
	* Chemical ionization (CI)	Reagent gaseous ions
	Field ionization (FI)	High-potential electrode
Desorption	* Field desorption (FD)	High-potential electrode
	* Electrospray ionization (ESI)	High electrical field
	* Matrix-assisted desorption-ionization (MALDI)	Laser beam
	Plasma desorption (PD)	Fission fragments from ^{252}Cf
	Fast atom bombardment (FAB)	Energetic atomic beam
	Secondary-ion mass spectrometry (SIMS)	Energetic beam of ions
	Thermospray ionization (TS)	High temperature

Molecular mass spec ion source considerations

- **Gas-phase**
  - Thermally stable compounds with bp less than 500^{o}C
  - Limited to masses less than 1000 Da (atomic mass unit; amu)
- **Desorption**
  - Does not require volatilization
  - Analytes up to 100,000+ Da

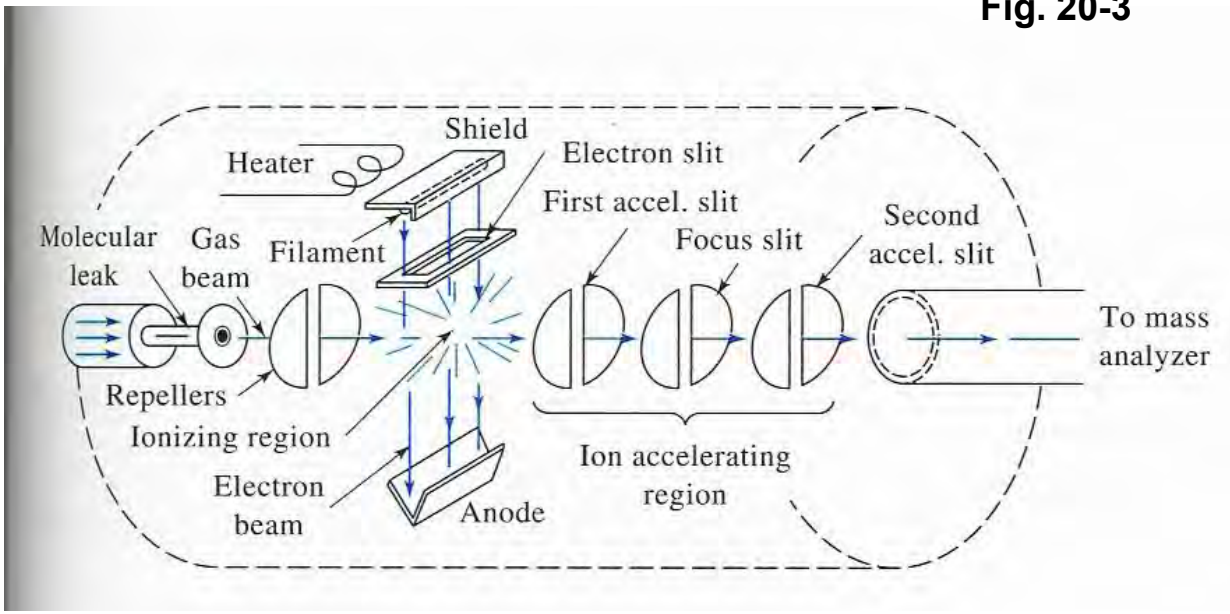
- **Hard vs. soft sources**
  - Hard sources leave molecule in excited energy states which relax via bond cleavage. Give “daughter ion” fragments at lower m/z.
  - Soft sources minimize fragmentation. Resulting spectra has fewer peaks.

The electron impact source

- Gas phase source: classical method still used today
- Sample is thermally vaporized and bombarded with beam of electrons
- Electrons are expelled from molecule due to electrostatic repulsion
  - Result: singly charged positive ions (primarily)

$$M + e^- \rightarrow M^{\bullet +} + 2e^-$$

Fig. 20-3



The electron impact source, continued

- Highly inefficient process and often gives complex spectra with many fragmented species
  - Daughter ions
- But, good sensitivity
- Most common ion source in (small) molecular mass spec

Fig. 20-4

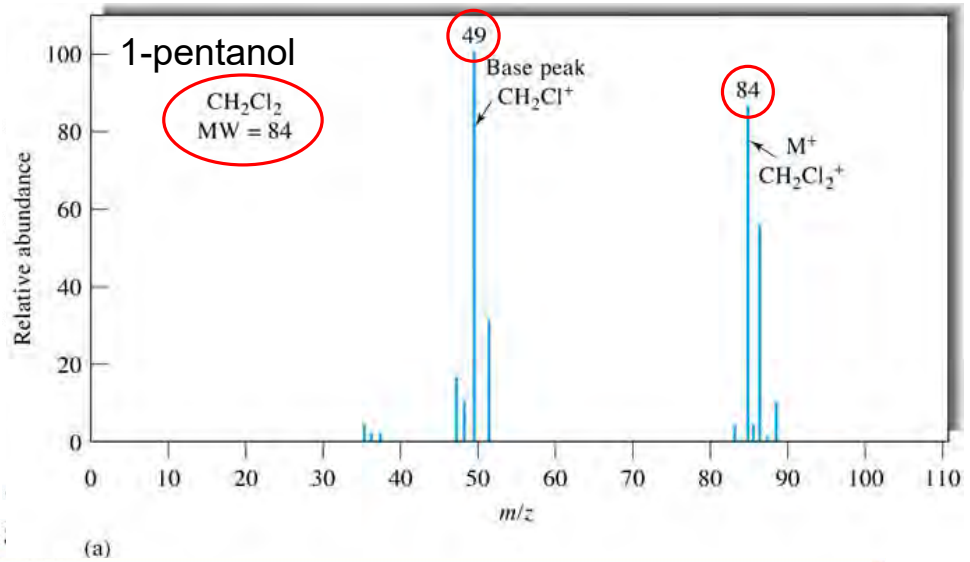
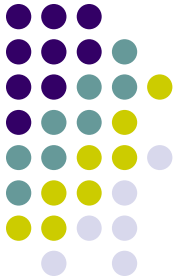


TABLE 20-2 Some Typical Reactions in an Electron-Impact

Molecular ion formation	$ABCD + e^- \rightarrow ABCD^{\bullet +} + 2e^-$
Fragmentation	$ABCD^{\bullet +} \rightarrow A^+ + BCD^\bullet$
	$\rightarrow A^\bullet + BCD^+ \rightarrow BC^+ + D$
	$\rightarrow CD^\bullet + AB^+ \rightarrow B + A^+$ or $A + B^+$
	$\rightarrow AB^\bullet + CD^+ \rightarrow D + C^+$ or $C + D^+$
Rearrangement followed by fragmentation	$ABCD^{\bullet +} \rightarrow ADBC^{\bullet +} \rightarrow BC^\bullet + AD^+$ or $AD^\bullet + BC^+$
Collision followed by fragmentation	$ABCD^{\bullet +} + ABCD \rightarrow (ABCD)_2^{\bullet +} \rightarrow BCD^\bullet + ABCDA^+$

Chemical ionization sources



- Gas phase source; M = molecule of interest
- Gas phase atoms of the sample are collided with ions generated from electron bombardment of a reagent gas
  - Reagent gas is often CH_4, which is ionized to CH_4^+, CH_3^+, and CH_2^+.

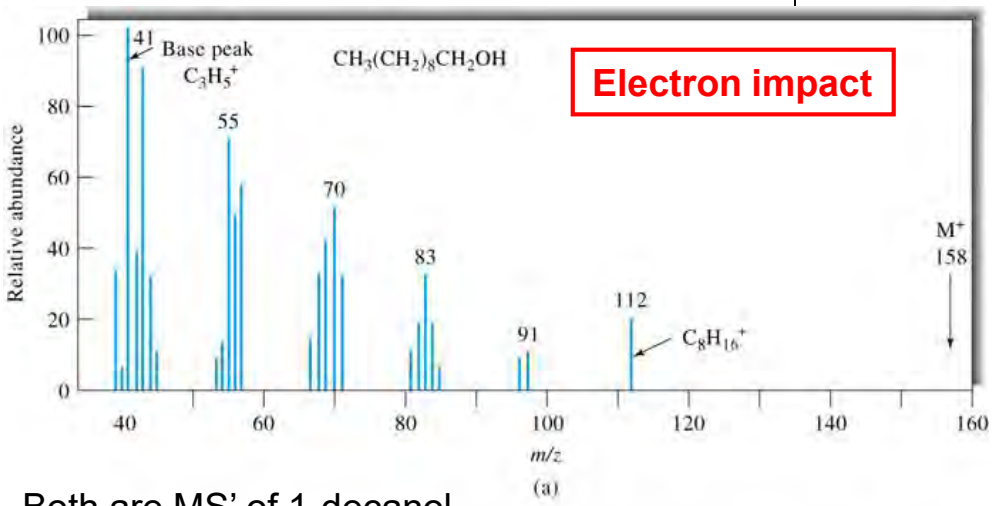
$$CH_4^+ + CH_4 \rightarrow CH_5^+ + CH_3$$
$$CH_3^+ + CH_4 \rightarrow C_2H_5^+ + H_2$$

- Ionization of sample typically occurs via proton transfer (H^+) or hydride transfer (hydride is H^-)

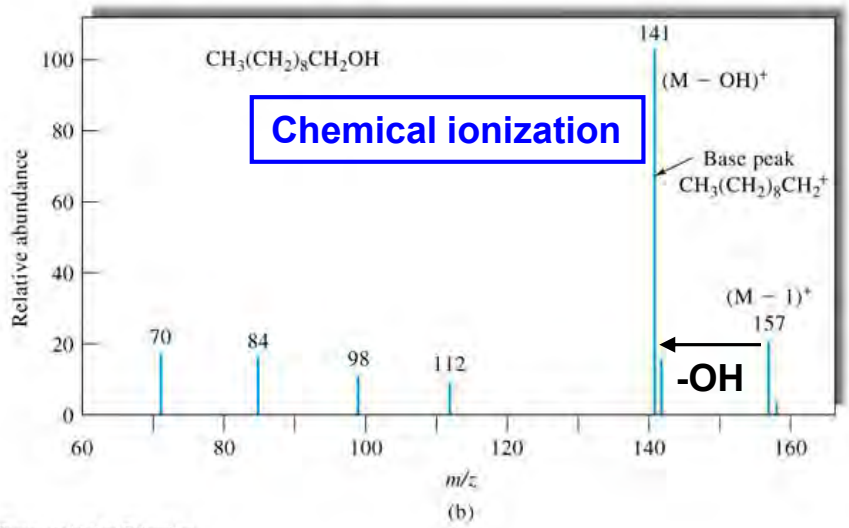
$$CH_5^+ + MH \rightarrow MH_2^+ + CH_4$$
$$C_2H_5^+ + MH \rightarrow MH_2^+ + C_2H_4$$
$$C_2H_5^+ + MH \rightarrow M^+ + C_2H_6$$

Comparison of electron impact and chemical ionization sources

- **Electron impact** is a much "**harder**" ionization method.
- Chemical ionization is "**softer**"—but still classifies as a "hard" method.
- Fragmentation can be an advantage or disadvantage; can add certainty or uncertainty.
- Both are limited to volatile and thermally-stable compounds



Both are MS' of 1-decanol



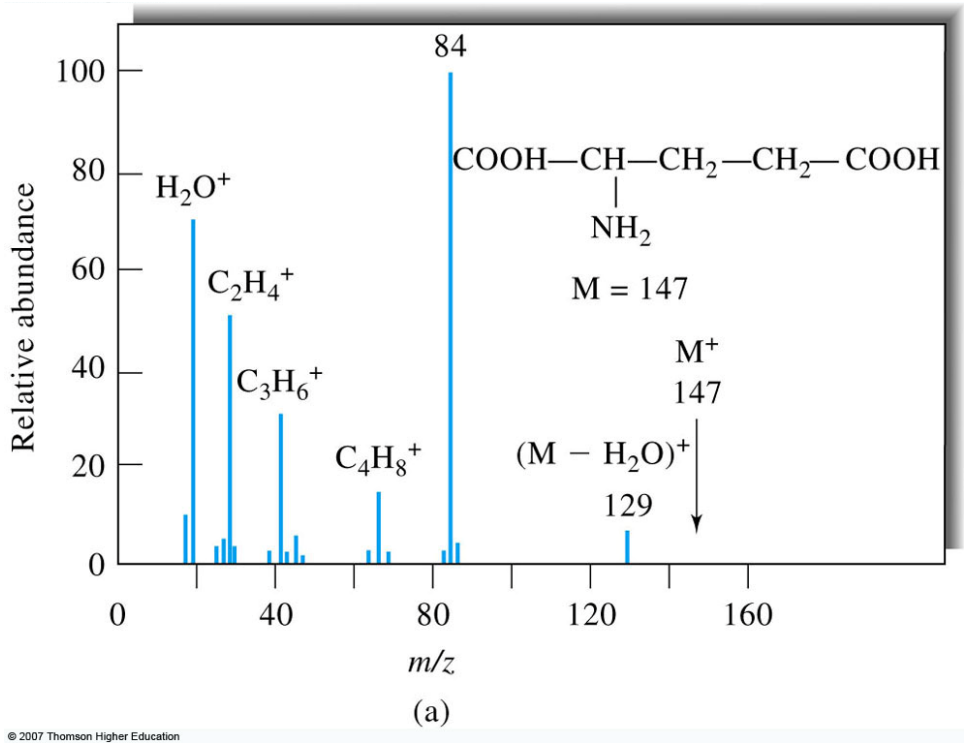
Field desorption

- Usually a **soft** ionization method
- Multi-tipped electrode is coated with sample solution (solid or liquid phase, not gas)
- Electrode is inserted into compartment and a high voltage is applied, and maybe heat
- The sample ionizes and goes into the gas phase by electrostatic repulsion; it desorbs from the surface it was on

Hard vs. Soft ionization

- Mass spectra of glutamic acid via different ionization sources

Electron impact



Field desorption

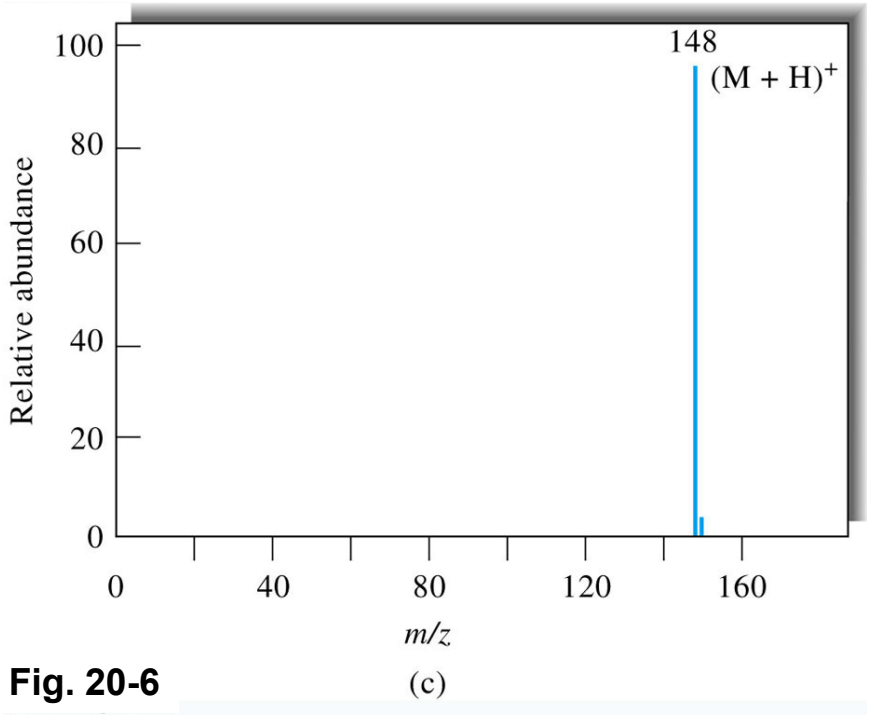


Fig. 20-6

Matrix-assisted laser desorption-ionization (MALDI)

- Demonstrated almost simultaneously in 1988 by German and Japanese research groups
  - Hillenkamp & Karas (Germany) and Tanaka (Japan)—Tanaka won the 2002 NP
- Sample is dispersed in matrix (usually a crystalline solid) and exposed to laser beam
- Matrix material absorbs radiation and "explodes" producing desorbed and ionized analyte molecules.
- Can analyze very large molecules *without* fragmenting; ***SOFT ionization***

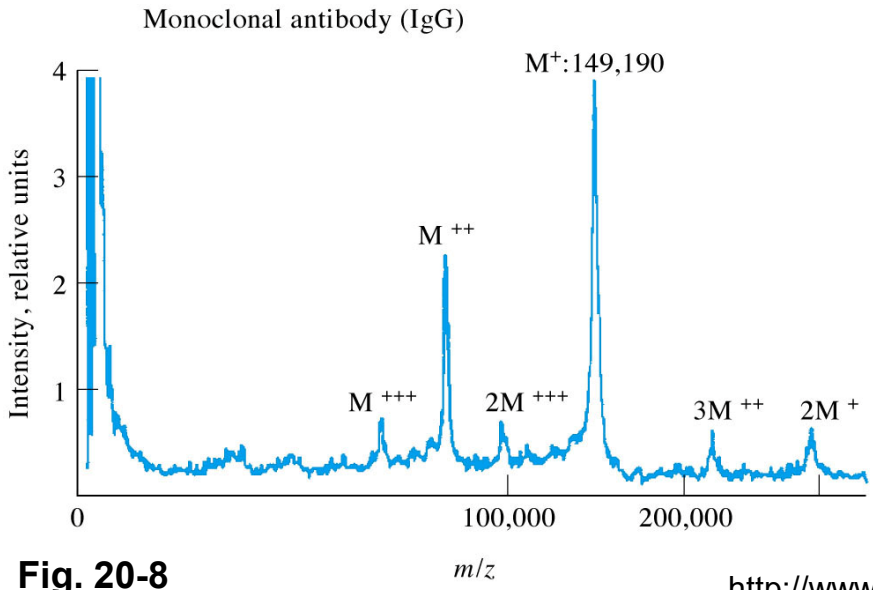
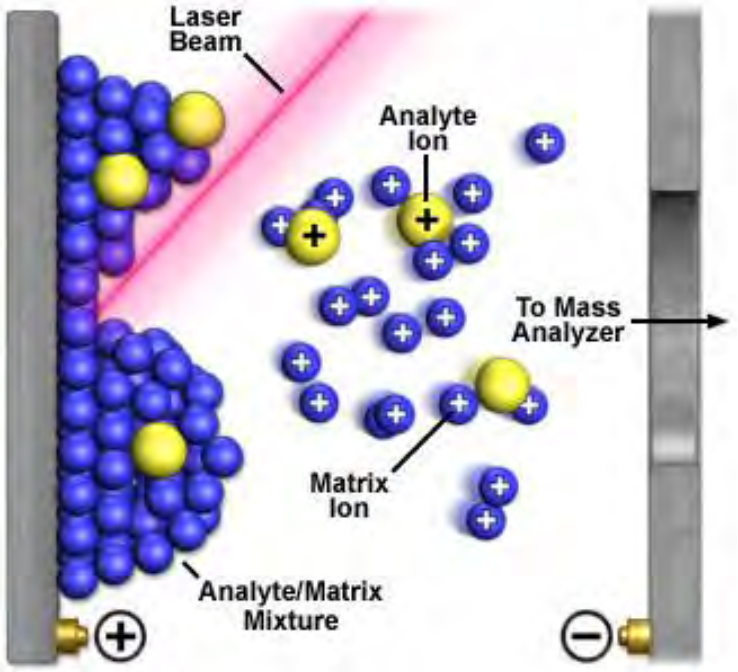
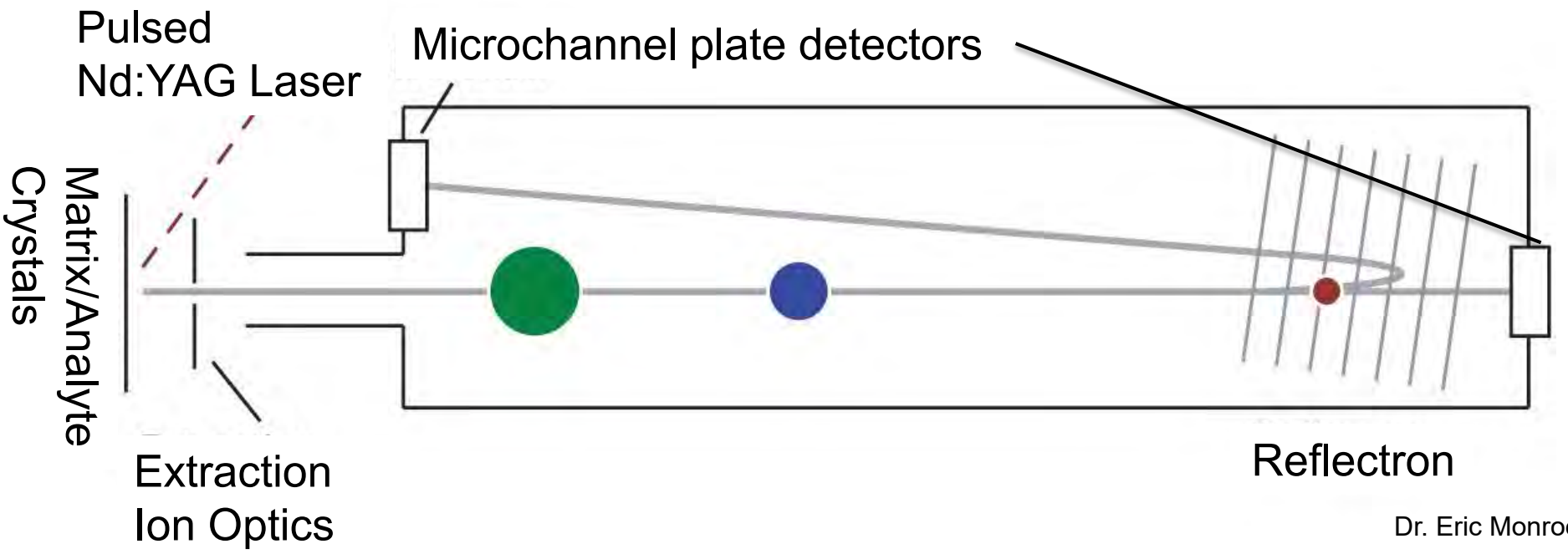


Fig. 20-8

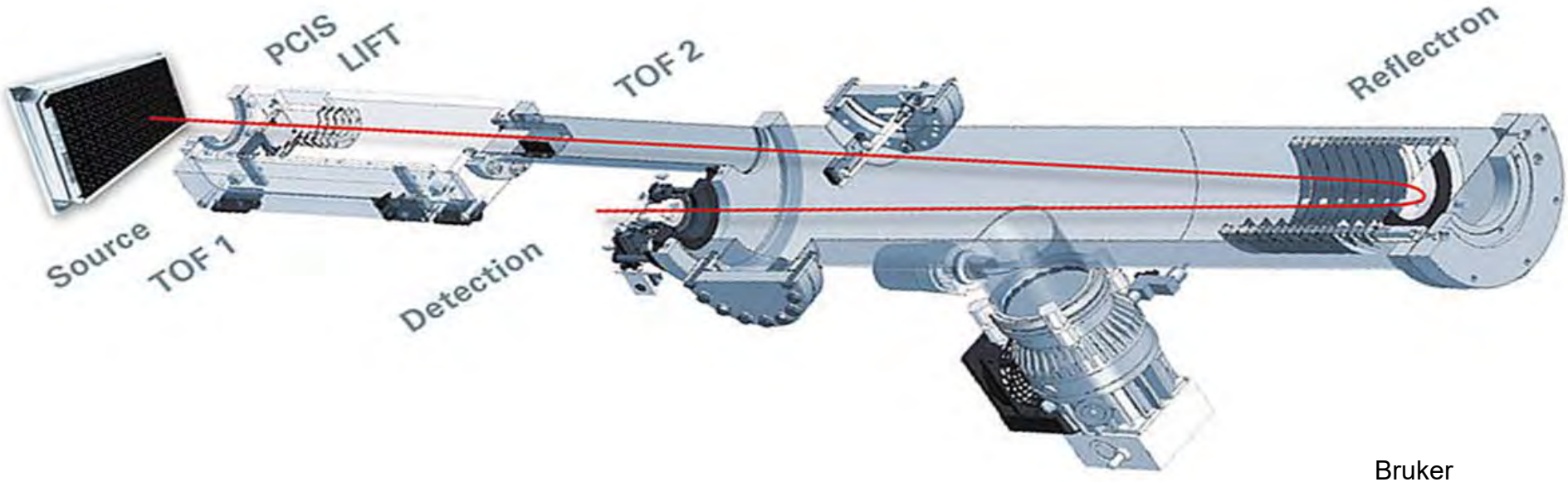


http://www.magnet.fsu.edu/education/tutorials/tools/ionization_maldi.html

Matrix Assisted Laser Desorption/Ionization

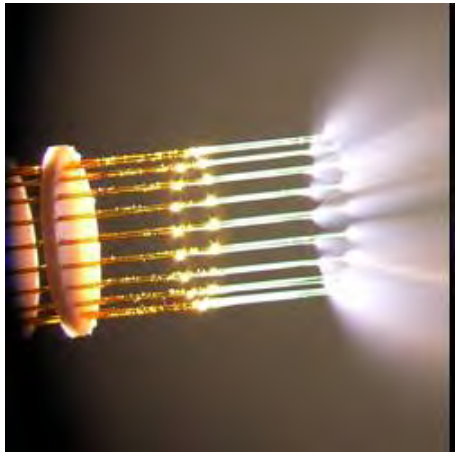


Dr. Eric Monroe



Bruker

Electrospray ionization (ESI)

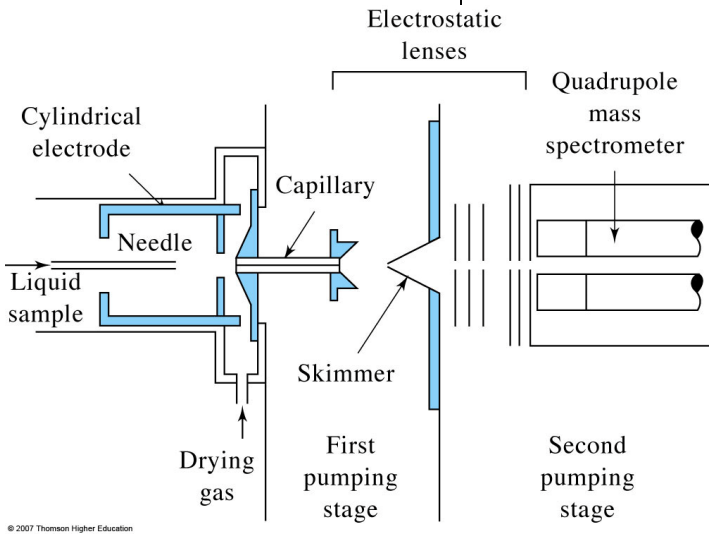


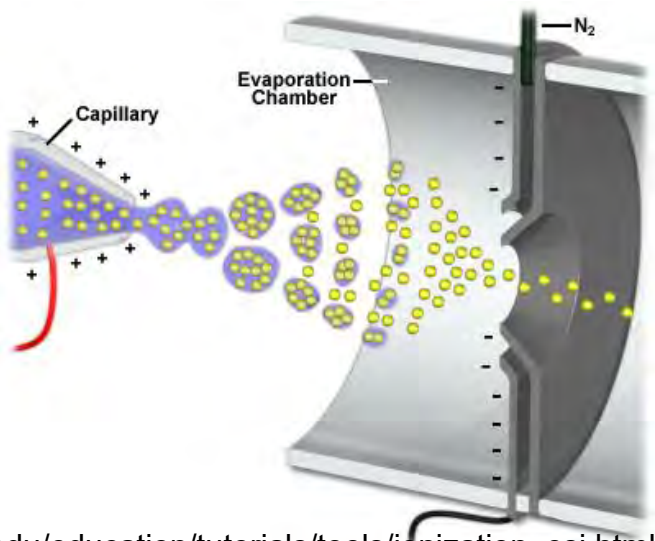
- Desorption method
- First described in 1984
- John Fenn (Virgina Commonwealth University) awarded 2002 Nobel Prize in Chemistry
  - With Koichi Tanaka (Shimadzu) for soft laser desorption (precursor to MALDI)
- Extremely useful for characterizing large biopolymers (oligonucleotides, peptides, proteins, etc.) having masses greater than 100,000 Da.
- Also very useful for inorganic and synthetic polymer research.

http://www.rdmag.com/RD100-Awards-Two-Techs-In-One-Yields-High-Flow-High-Ionization/

Electrospray ionization (ESI): How it works

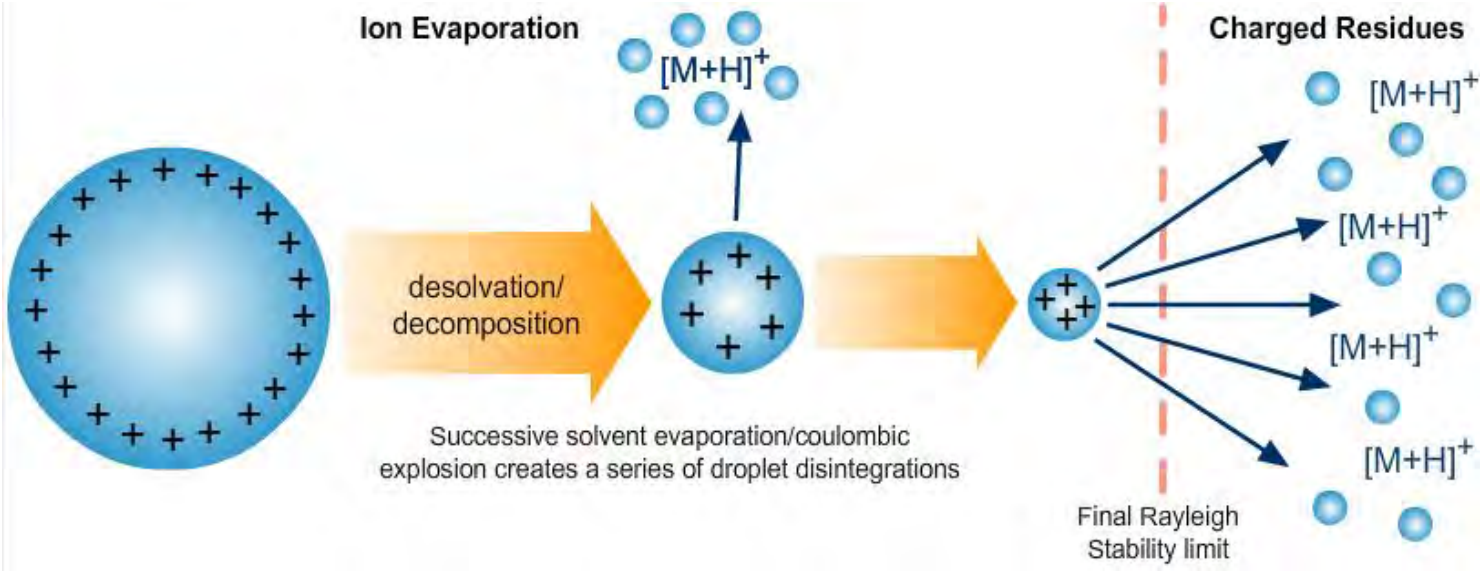
- Sample (in solution) passed through needle biased at several kV
- Charged droplets pass through capillary where solvent evaporates and molecules begin to inherit the droplets charge
- Droplets shrink until their surface tension can no longer support the charge density—Rayleigh limit
- Coulombic explosion occurs and the droplet bursts into smaller droplets that repel each other
- Process repeats until all of the solvent in gone and analytes are multiply charged.
- A *very big deal* because it couples flowing, liquid samples to mass spec!

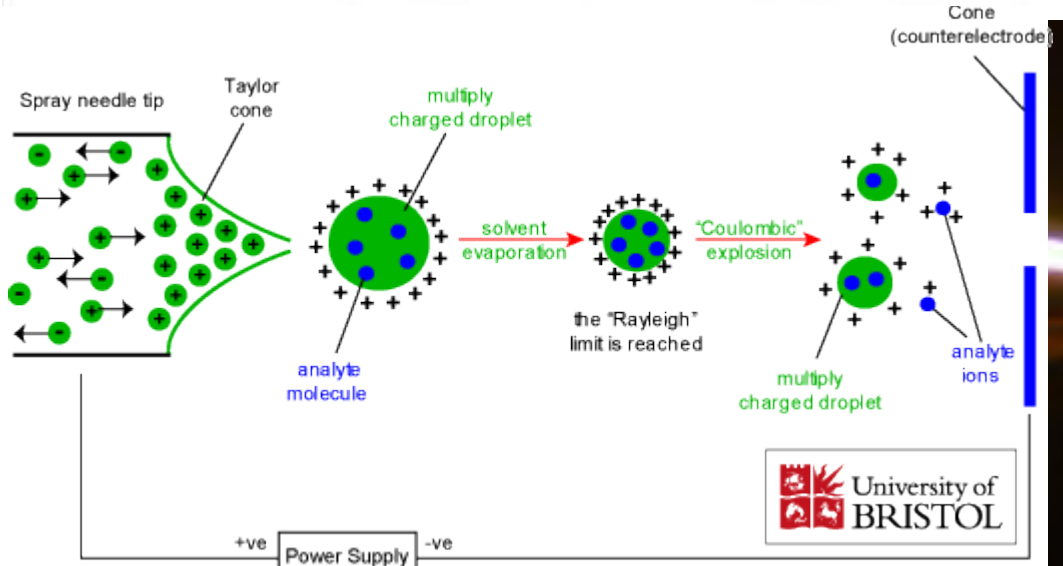


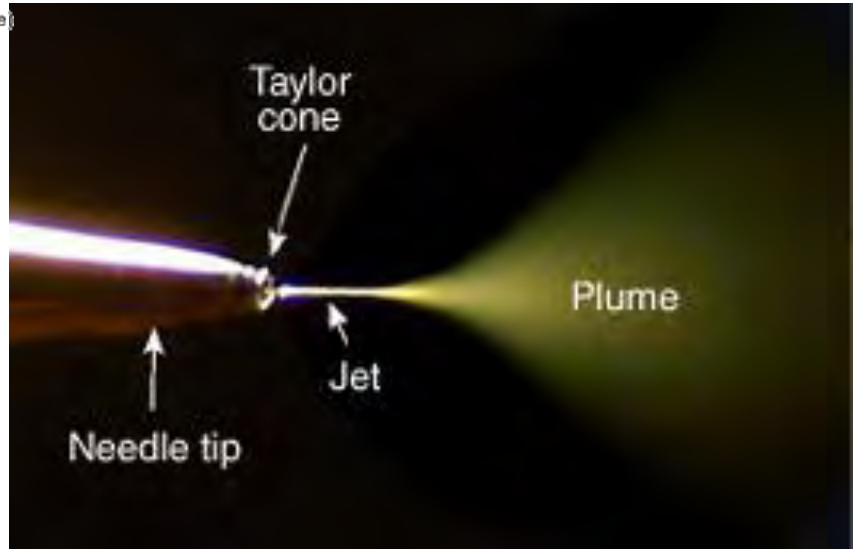


http://www.magnet.fsu.edu/education/tutorials/tools/ionization_esi.html

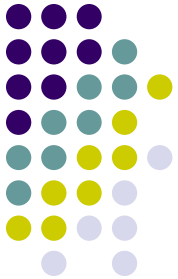
ESI: A few more images







Electrospray ionization (ESI), continued

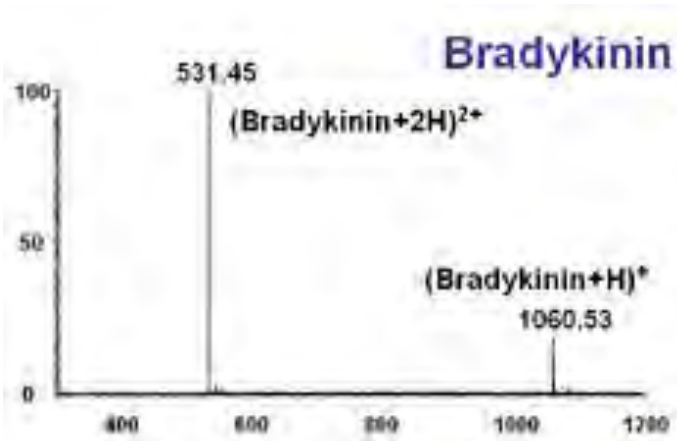


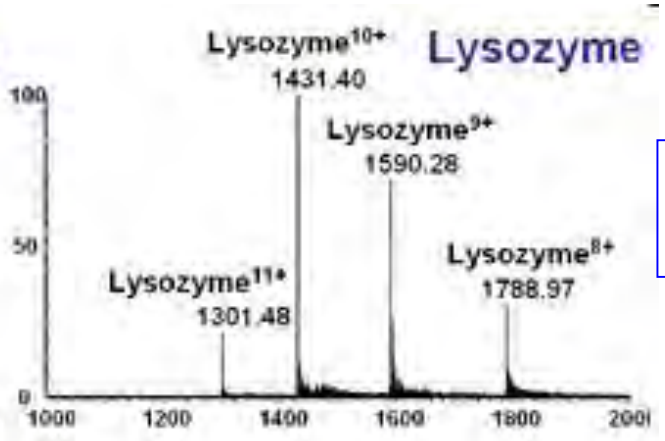
- **Multiply charged** analytes have low m/z ratios
  - Typically less than 1500; interfaces with quadrupole mass analyzer; more later
- Molecular mass can be determined from isotopic peak distribution (back calculation)
- No fragmentation – disadvantage in some applications
  - Use MS/MS (mass spec followed by mass spec again) to gain more structural knowledge of the molecule
- Readily interfaces with solution-phase
  - Takes place at atmospheric pressures and temps (RT)
  - Liquid chromatography and capillary electrophoresis

ESI-MS Data

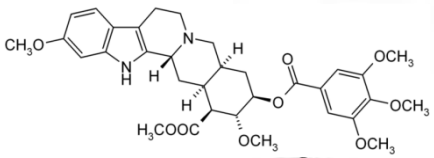
- Note multiple charges and low m/z ratios
- Large, complex, complete molecules

MW = 1060 Da



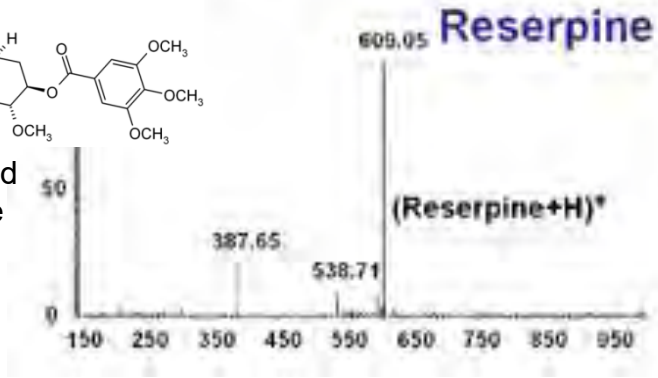


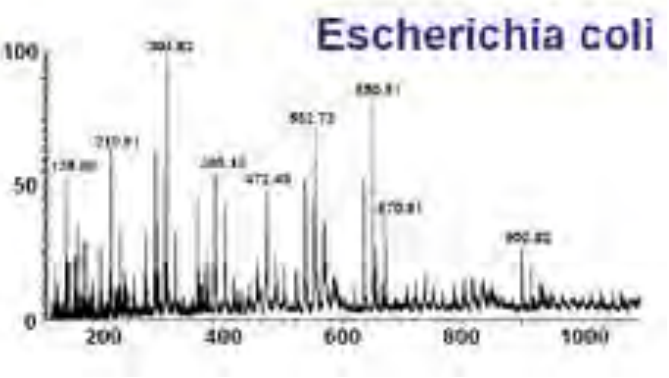
MW = 14,700 Da



antipsychotic and antihypertensive

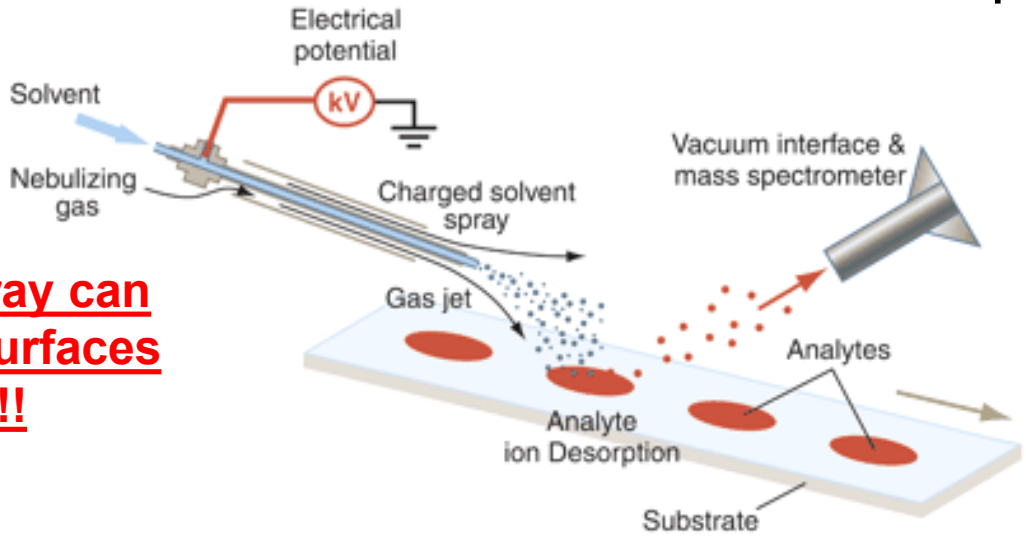
608.68 g/mol





Desorption Electrospray Ionization (DESI)

- Method developed by Prof. Graham Cooks (Purdue)
  - "Mass Spectrometry Sampling Under Ambient Conditions with Desorption Electrospray Ionization", *Science*, **2004**, *306*, 471-473.
- A fine spray of charged droplets hits the surface of interest, from which it picks up small organic molecules and large biomolecules, ionizes them, and delivers them—as desolvated ions—into the mass spectrometer



Now electrospray can be applied to surfaces and interfaces!!!

R.G. Cooks, Z. Ouyang, Z. Takats, and J.M. Wiseman, *Science,* **2006,** *311*, 1566-1570.

After generating ions …

- **Sorting** ions by mass
- **Detection** of ions

- **Single-channel** detectors
  - Faraday cup
  - Electron multiplier
- **Array transducers** for ions
  - Microchannel plates
  - Micro-Faraday array
    - microfabricated array of Faraday cups

Faraday cup

- The charge from positive ions striking plate is neutralized by electrons supplied from ground by external circuit via a load resistor.
- The voltage dropped across the resistor can be amplified and is proportional to the number of ions that struck the collector.
- The Faraday cage eliminates reflections of ions or secondary electrons ejected from collector.
- Response is independent of energy, mass, chemistry, etc. A **universal charge detector**.
- Inexpensive and simple design. High impedance amplifier limits time resolution. But, not as sensitive as electron multiplier (next).

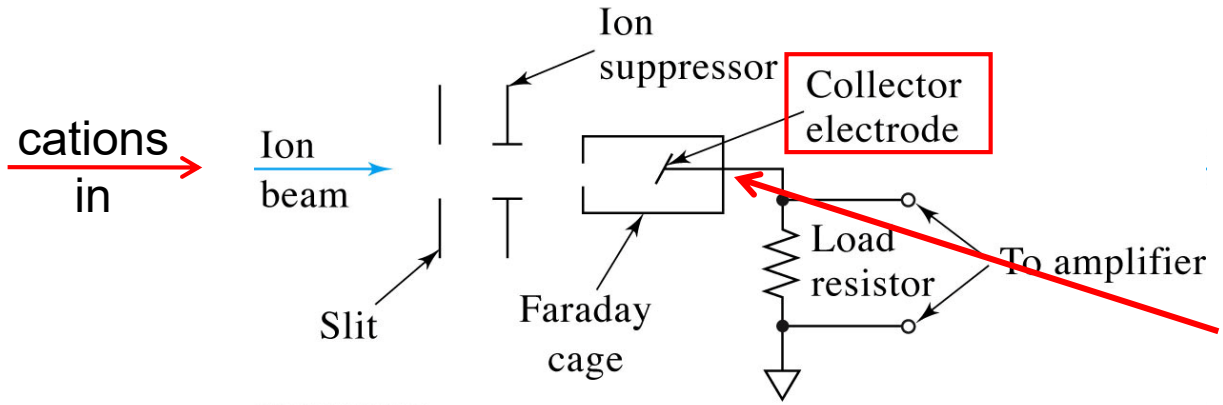
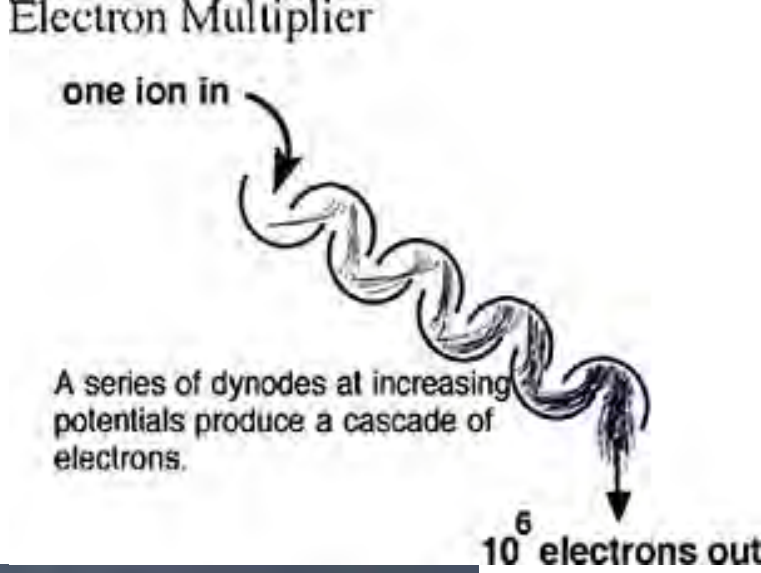
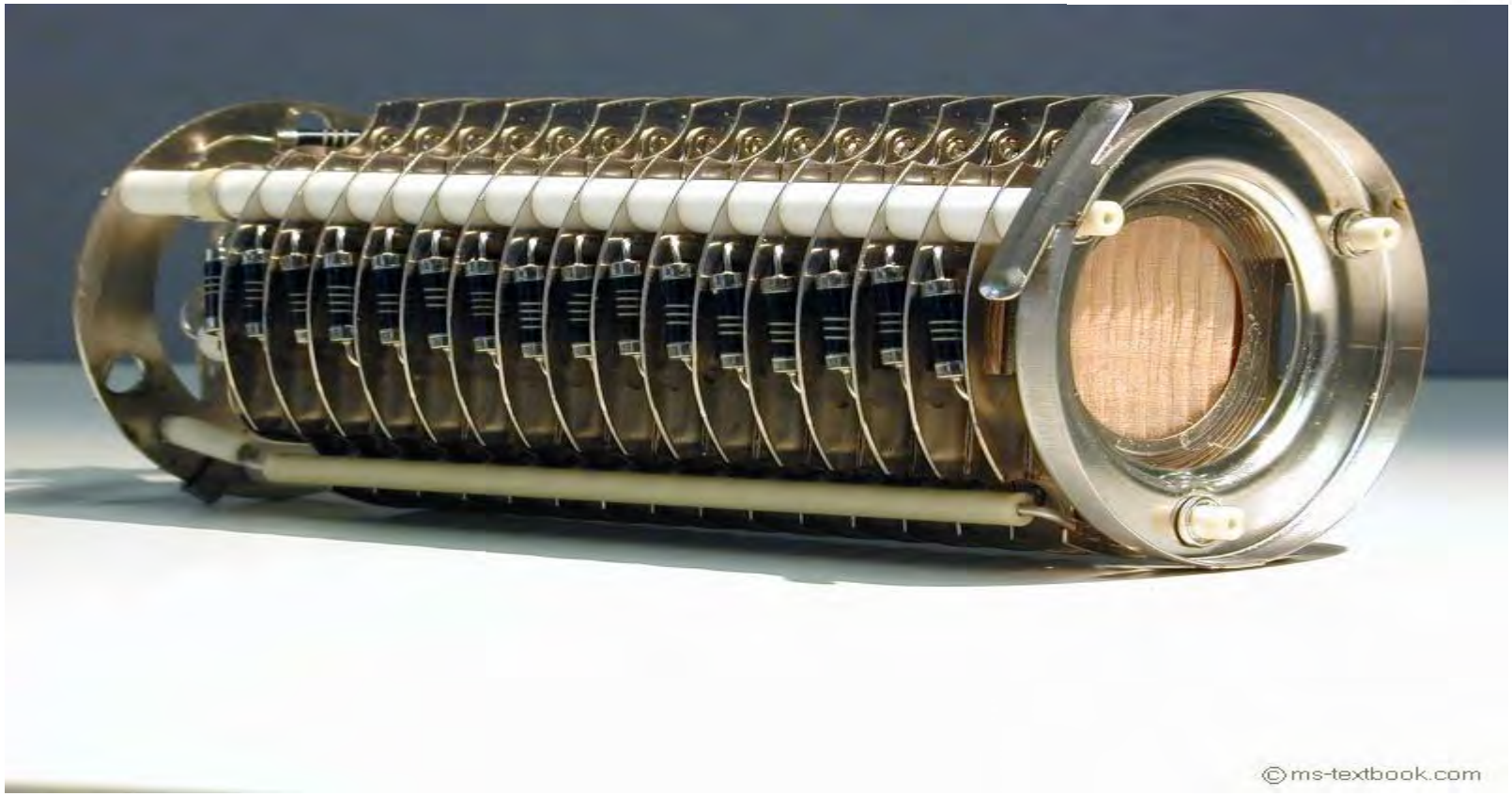


Fig. 11-3

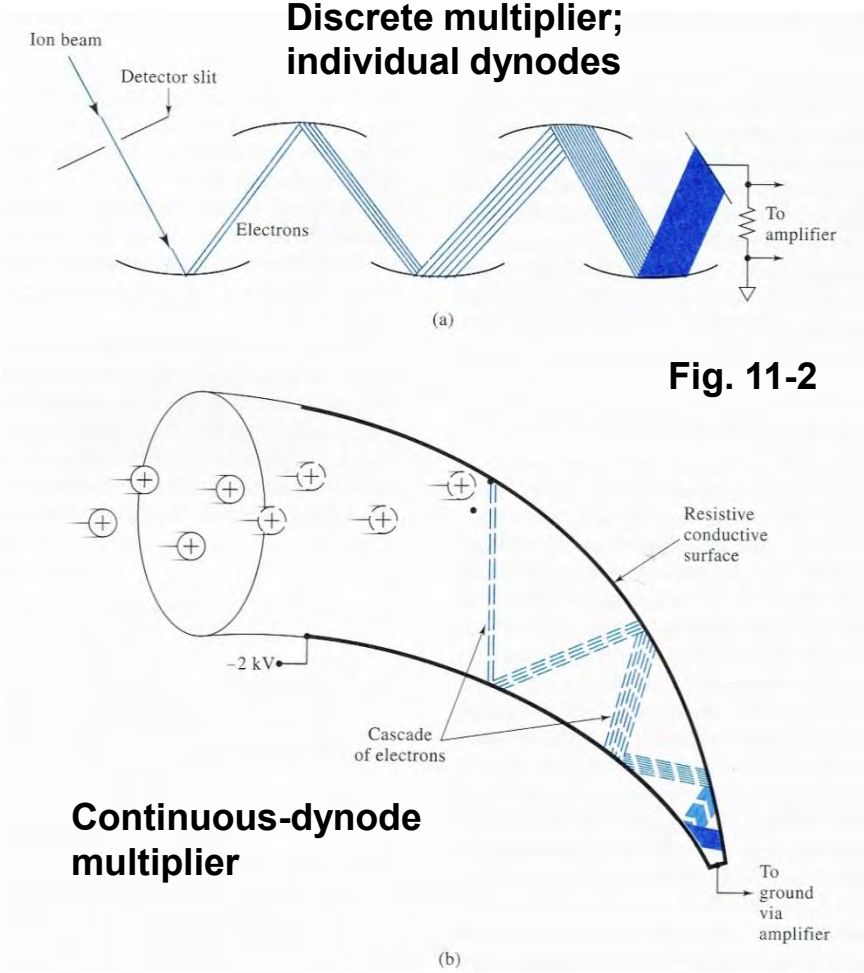
Electron Multipliers



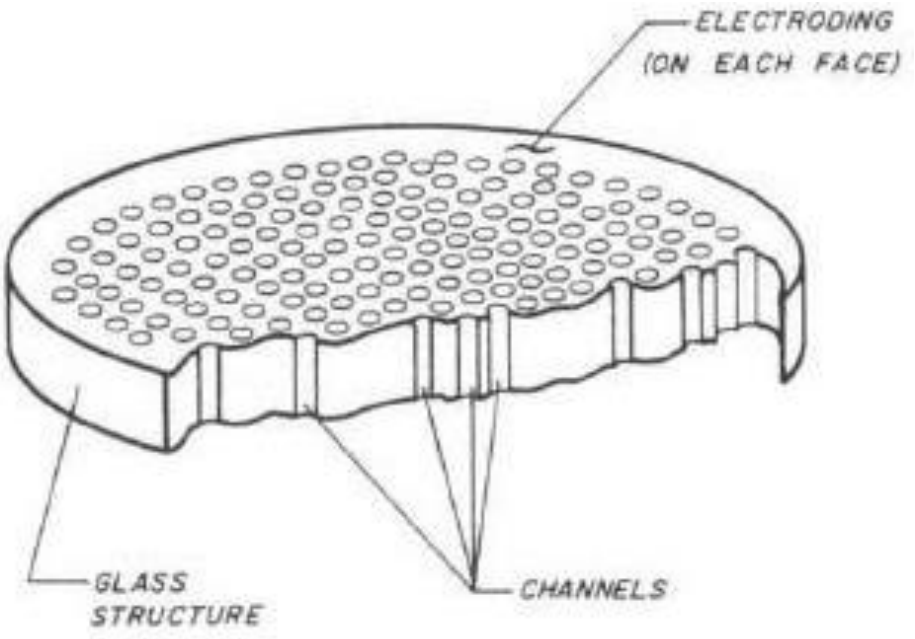


Electron multipliers

- Similar to photomultiplier tubes used in optical detection
- Ions strike cathode generating a burst of electrons that are subsequently amplified by a series of dynodes.
- Multipliers with ~20 dynodes can give gains of 10^8.
- Rugged, reliable, high-current gains with fast response times (nsec)
- Used for most routine mass spectrometry applications



Microchannel Plate detector



18mm

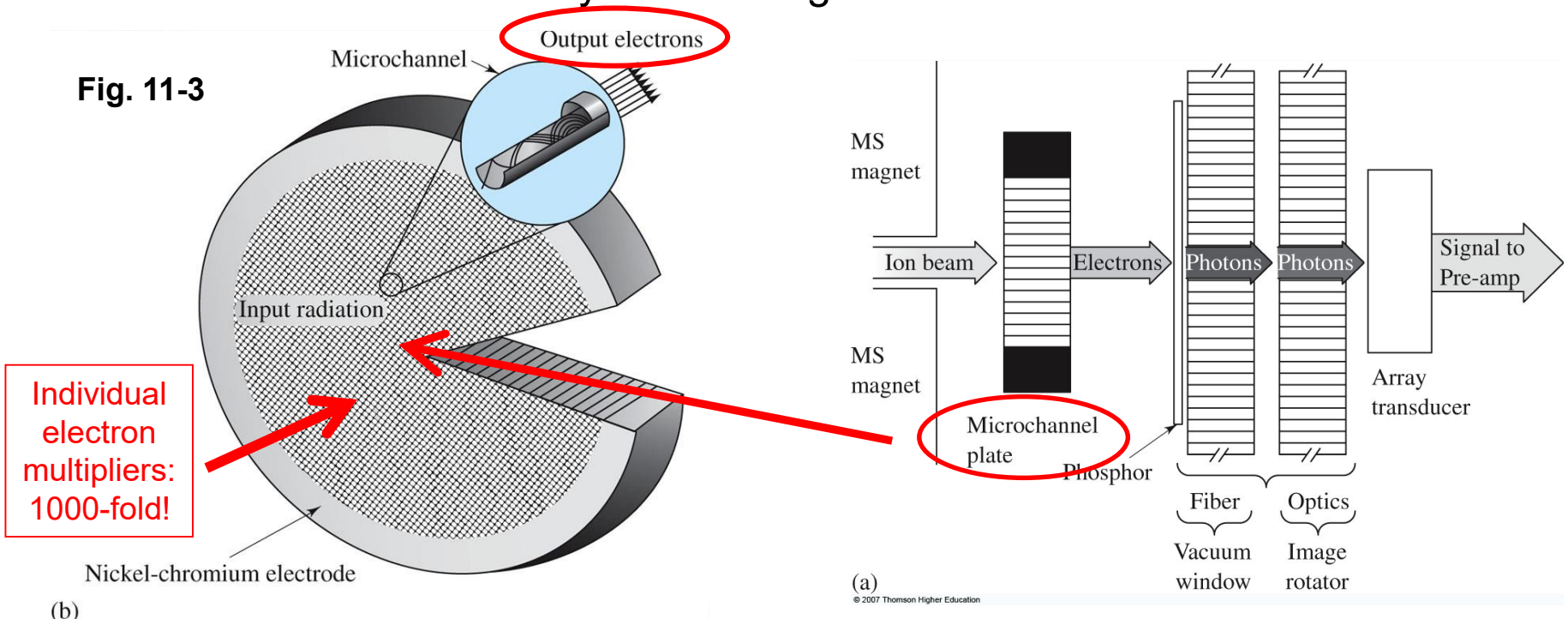
An MCP detector is like placing 1,000s of electron multipliers in parallel right next to each other.

They are typically used in time-of-flight instruments because of their exceptional speed and sensitivity.

Microchannel plate—an array detector for ions

- Electro-optical ion detector (electrons and light)
- Analogous to optical detectors, detector arrays allow multiple ion trajectories (different ions) to be monitored simultaneously
  - The utility of this type of detector will be discussed later
    - Think diode array versus single diode

Fig. 11-3



Mass analyzers

- Sort ions based upon *m/z* ratio
  - Exert forces upon ions in order to differentiate m/z ratios
  - Ions can be manipulated by electric and magnetic fields.
  - Ions that traverse a magnetic field have a bent trajectory
- Efficiency of ion sorting described by **resolution**:

$$R = \frac{m}{\Delta m}$$

Δm is the mass difference between the two peaks
m is the average mass of the peaks

- Common types
  - **Magnetic sector**
  - **Double-focusing**
  - **Time-of-flight**
  - **Quadrupole**
  - **Quadrupole ion trap**
  - **FT-Ion cyclotron resonance**
  - **Orbitrap**

Needed resolution varies dramatically based upon application!!!

Better *R* often means more $ or more complex instrumentation!

Magnetic sector analyzers

- Ions are injected into a circular path (electro)magnet and only those of a certain *m/z* ratio are able to balance the centripetal (F_C) and magnetic forces (F_M) in successfully traverse the path; others hit the sides. ***Assumption: all ions have same KE after acceleration through Slit B***
- Mass selection is achieved by sweeping the field, *B* (more typical) or *V*.
- Resolution ≤ 2000

http://www.magnet.fsu.edu/education/tutorials/java/singlesector2/index.html

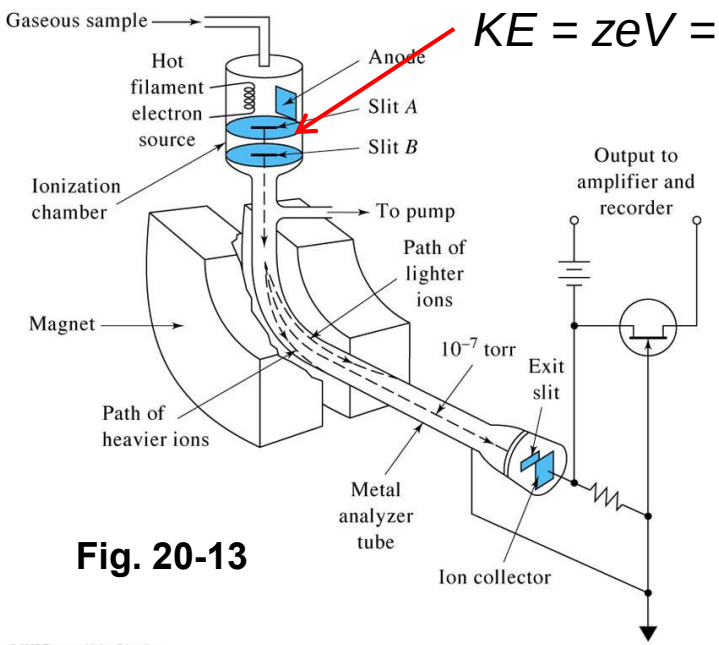


Fig. 20-13

$KE = zeV = ½\ mv^2$

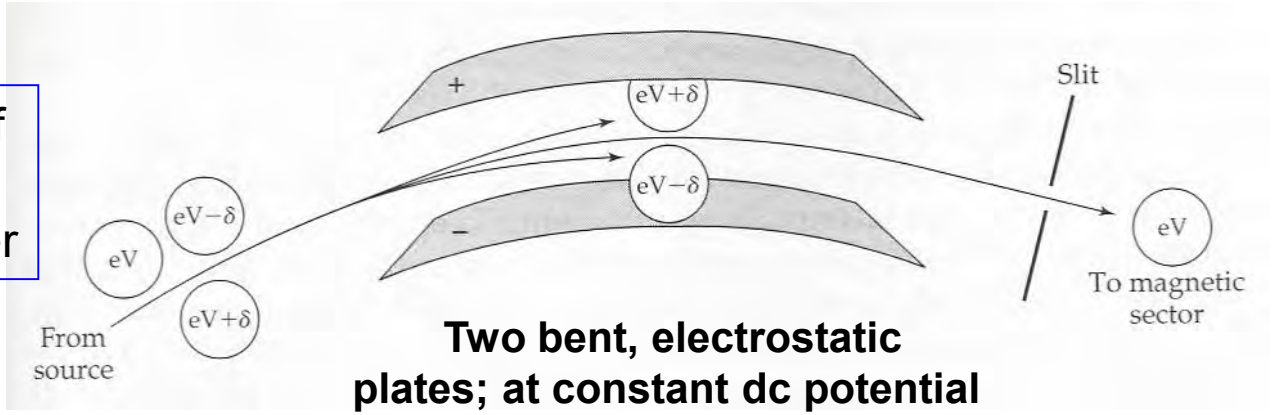
$$F_M = Bzev \qquad F_c = \frac{mv^2}{r}$$

B=magnetic field strength *e*=electron charge
v=velocity *V*=accelerating voltage
r = radius m = mass

Limitations of magnetic sector

- Not all ions of same *m/z* have identical kinetic energy
  - A Boltzmann distribution emerges from ion source
  - Causes broadening in *m/z* transmitted to detector
- Solution: Use a second focusing element before magnetic sector—"double focusing"

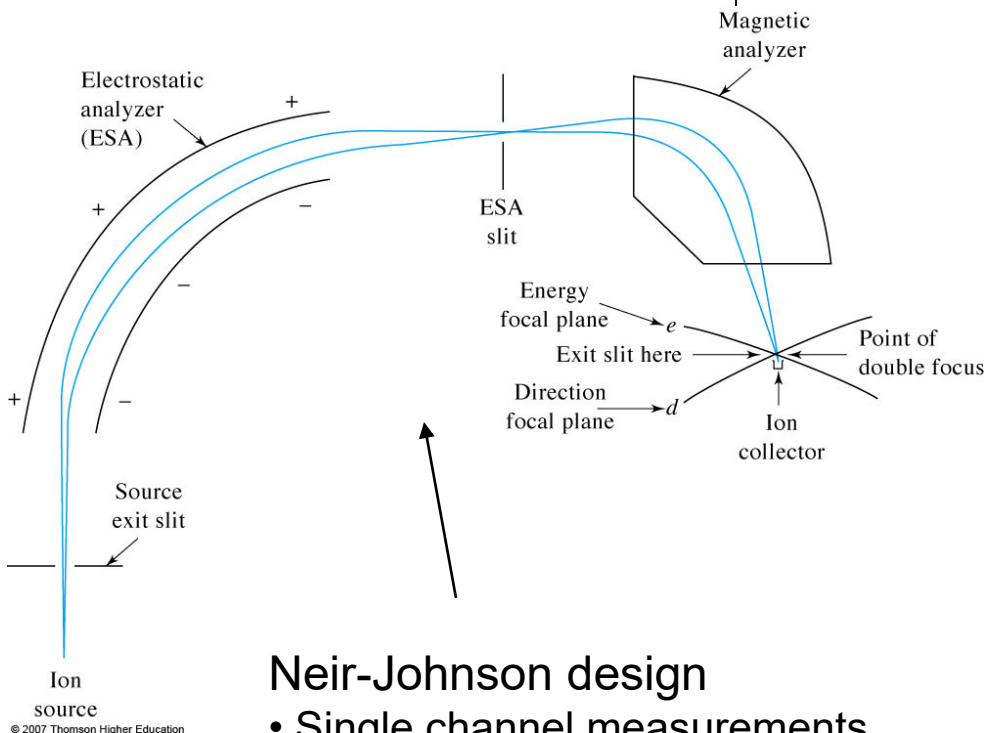
Kind of like a pre-filter



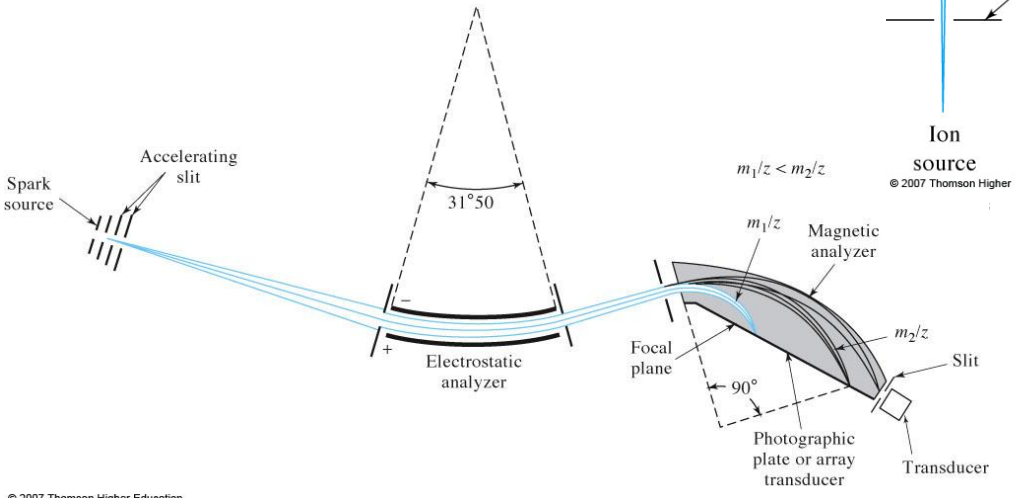
Two bent, electrostatic plates; at constant dc potential

Double focusing analyzer

- Ions are first passed through electrostatic energy filter to isolate a single kinetic energy
- Resolution ≥ 100,000!



Neir-Johnson design
- Single channel measurements
- Early design



Mattauch-Herzog configuration
- Better for array detectors that capture entire mass spectra

http://www.magnet.fsu.edu/education/tutorials/java/dualsector/index.html

Time-of-flight mass analyzer (TOF)

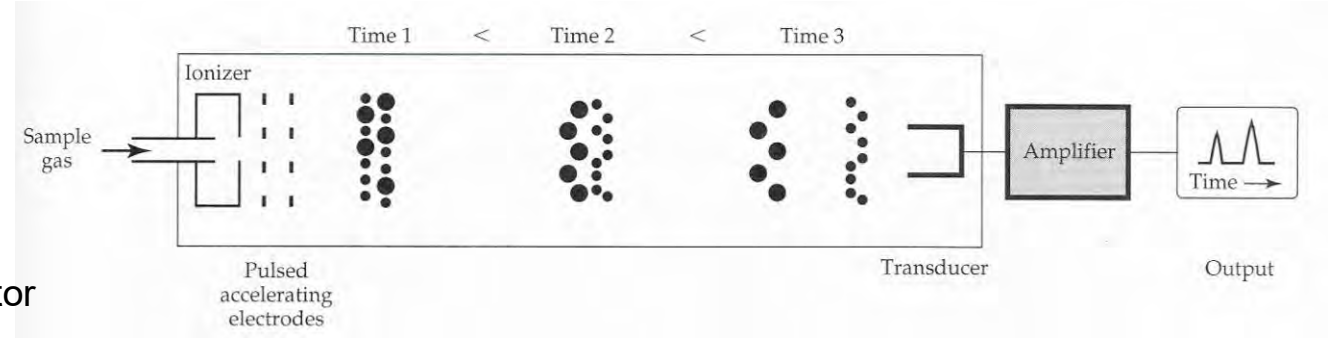
- Ions are generated in pulses and accelerated (also in pulses, but with a lag) in a 10^3-10^4 V field into a field-free drift tube
- All ions (theoretically) have the same kinetic energy so higher mass species will traverse the drift tube more slowly (big mass = slow v)

$$KE = \frac{1}{2}mv^2$$

- Resolution better than, but sensitivity (quantitation) not as good as quadrupoles (next).
- Advantages: simplicity, ruggedness, *virtually unlimited range*, and *fast data acquisition* (entire mass spectrum collected simultaneously)

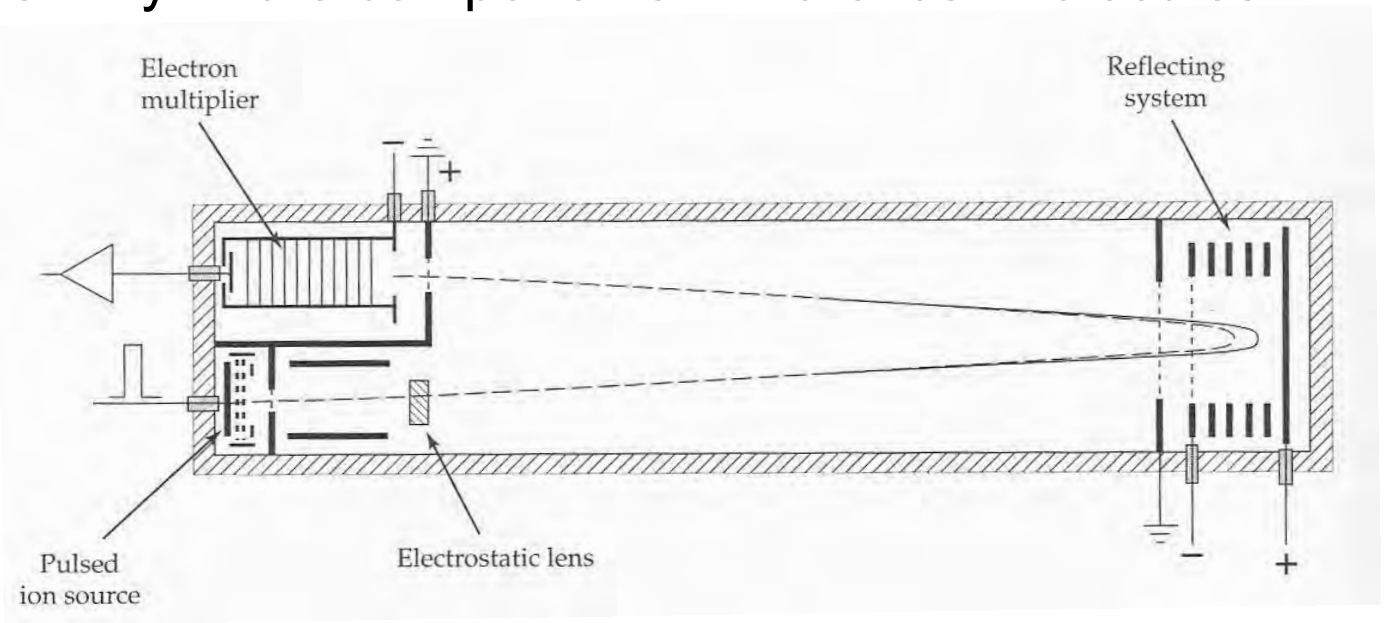
$$t_F = L \cdot \sqrt{\frac{m}{2Vez}}$$

t_F=flight time
L=distance from source to detector
V=accelerating voltage



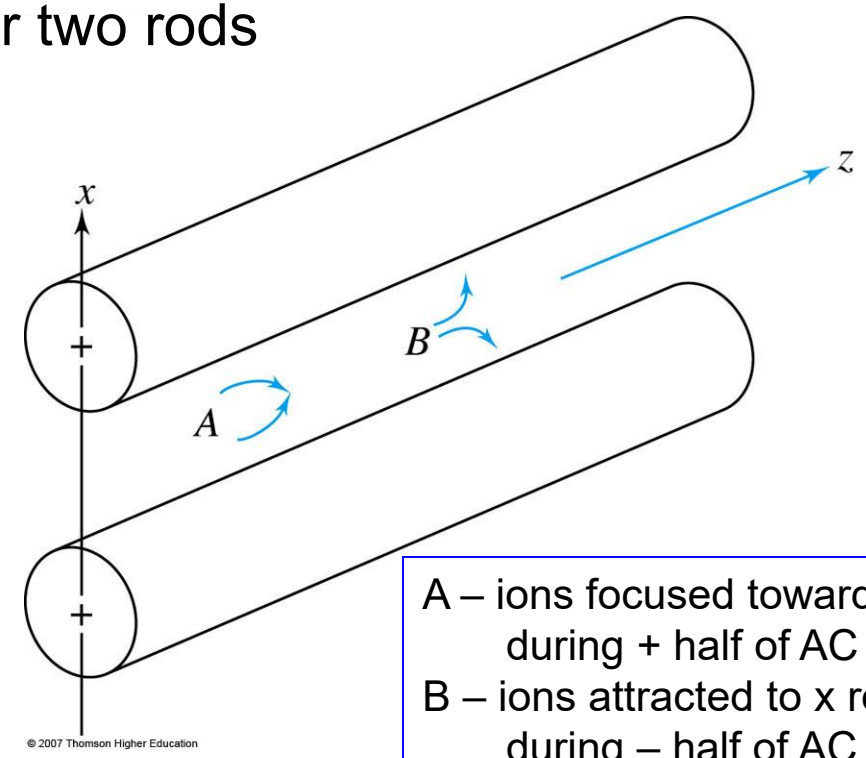
Reflectron configuration: a variation on standard TOF

- Faster ions penetrate more deeply into electrostatic reflector and thus travel a longer distance.
  - Correcting for KE distribution from ion source
- Result: Faster and slower *ions of same mass arrive at the detector simultaneously*. Improved resolution but lower sensitivity: more components = more lost molecules



Quadrupole mass analyzer

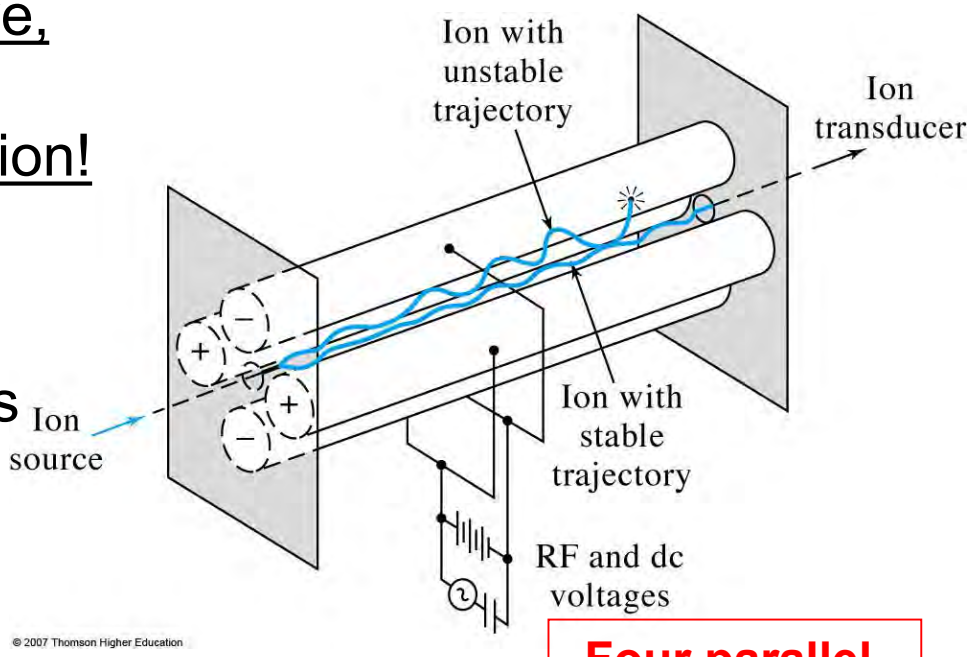
- Mass isolation is achieved with alternating applied electric fields
- DC voltage is applied to rods opposite one another and AC voltage is superimposed. The same thing, but opposite sign of applied voltage on the other two rods
- Important: The DC and AC voltages are both essential and are always controlled and scanned together.
- The repulsion (or not) of an ion is dependent upon its velocity which tracks with m/z. Limited, but tunable, mass range passes.



A – ions focused toward z-axis during + half of AC cycle
B – ions attracted to x rods during – half of AC cycle

Quadrupole mass analyzer, continued

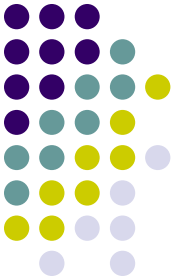
- Only ions having a specific *m/z* ratio are confined within and traverse the entire quadrupole without becoming unstable and colliding with a rod.
- Most commonly used mass analyzers today for a range of applications
- More compact, less expensive, and more rugged than TOF instruments—better quantitation!
- Good resolution (R ~ 1 amu)
  - Much less than TOF
- Fast! 100-ms scans, full mass spectra
- Nominal mass range is up to 1500 m/z
  - Best go up to 3000-4000 *m/z*.



Four parallel electrode rods

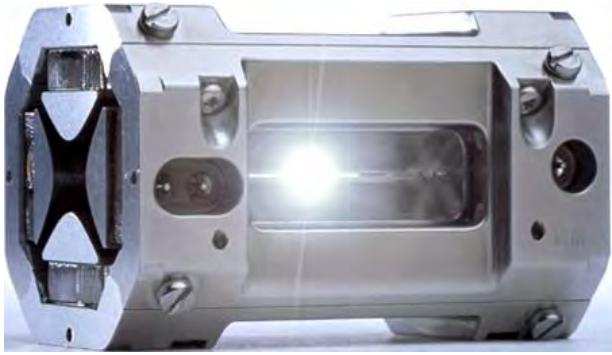
http://www.youtube.com/watch?v=8AQaFdI1Yow&feature

Putting it all together: Helpful video

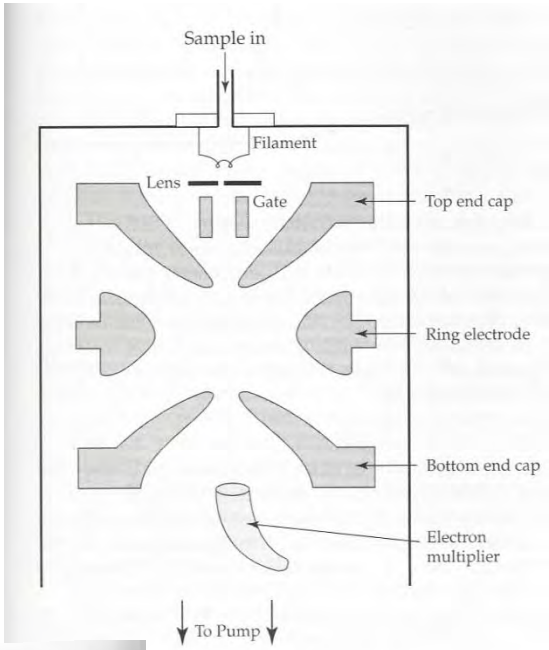


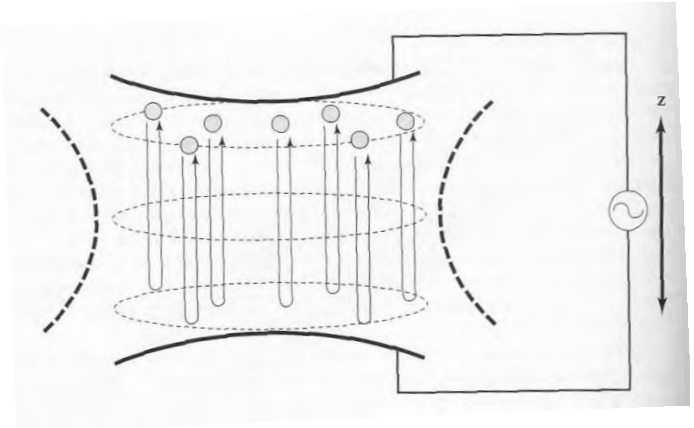
- Small molecule mass spectrometry
  - Ionization, quadrupole mass analyzer, and detector
  - http://youtu.be/WbX27Gg5ziU

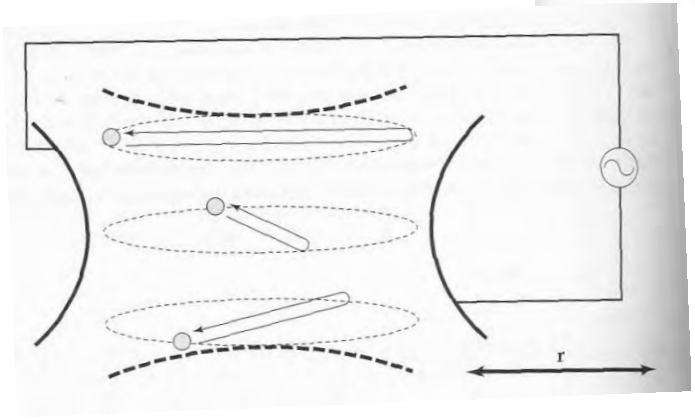
Quadrupole ion trap



- Can store ions of particular *m/z* ranges and selectively eject particular *m/z* ions for up to 15 minutes, for some species
- Rugged, compact, and cheap, but limited dynamic range and accuracy in *m/z* measurement. Useful for MS/MS and other applications where one wants to store or accumulate ions for subsequent analysis.

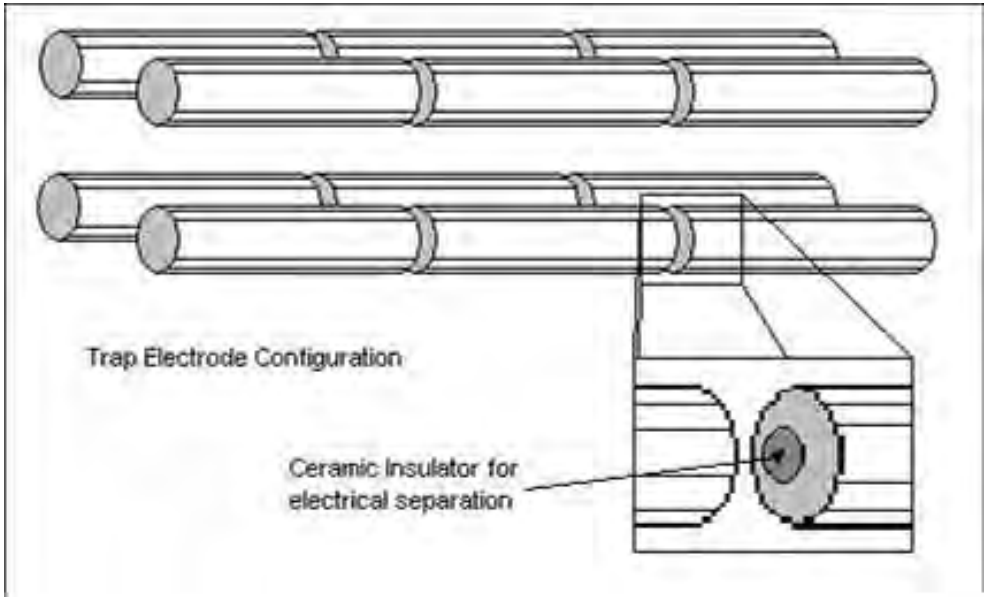






Linear ion trap

- Rods are segmented to allow independent electrical contact.
- Potential well created in middle traps ions
  - Higher capacity than standard quadrupole ion trap



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

Tandem mass spectrometry

- A pre-selected ion is fragmented and the pieces analyzed again by mass spectrometry.
  - Parent ion (M^{n+}) fragmented into product ions
  - Fragmented via:
    - Electron capture dissociation
    - Collisions with gas (often CH_4)
    - Photo-induced dissociation (w/laser)
  - Valuable tool for identification of species with the same parent ion mass because the fragments will differ
  - Soft ion sources preferable for MS step 1
  - Harder sources preferable for MS step 2

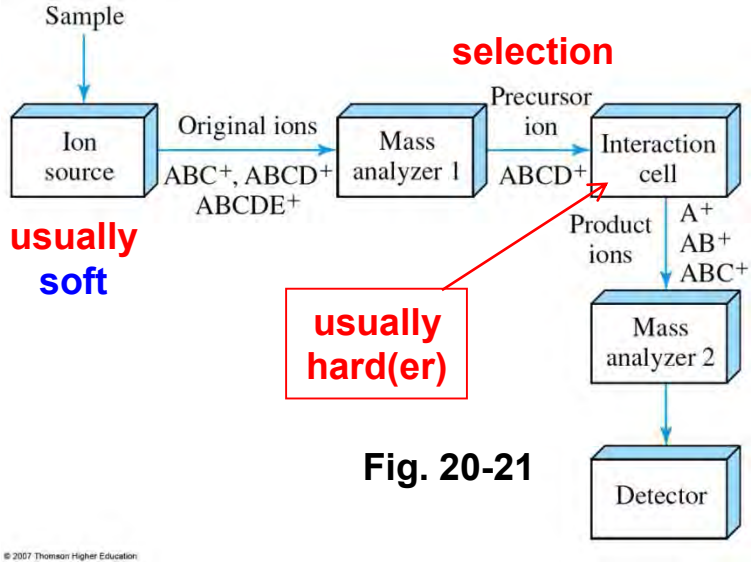


Fig. 20-21

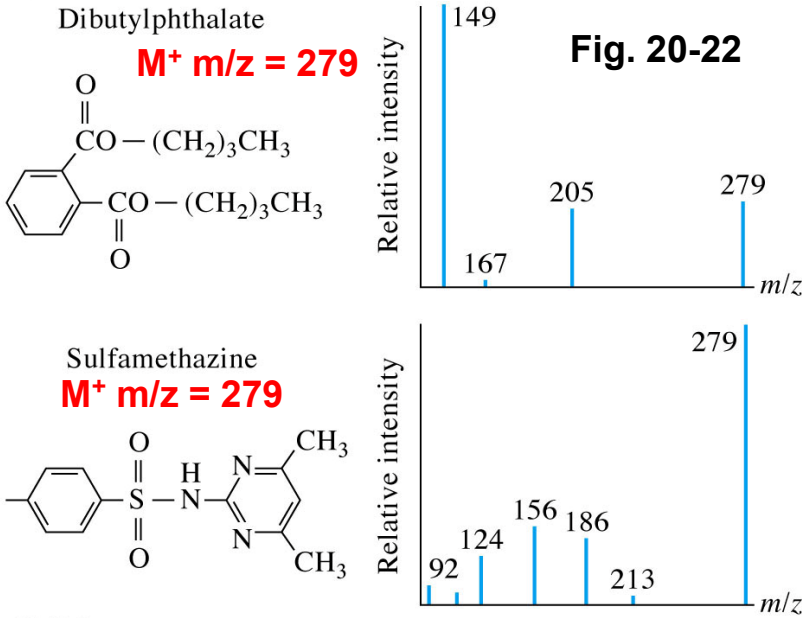


Fig. 20-22

Types of MS/MS instrumentation

- Tandem-in-space MS/MS
  - Often multiple quadrupoles that can act both as mass analyzers and collision chambers
  - Image below
- Tandem-in-time MS/MS
  - Collision is done inside of mass analyzer
  - FT-ICR and quadrupole ion traps are ideally suited

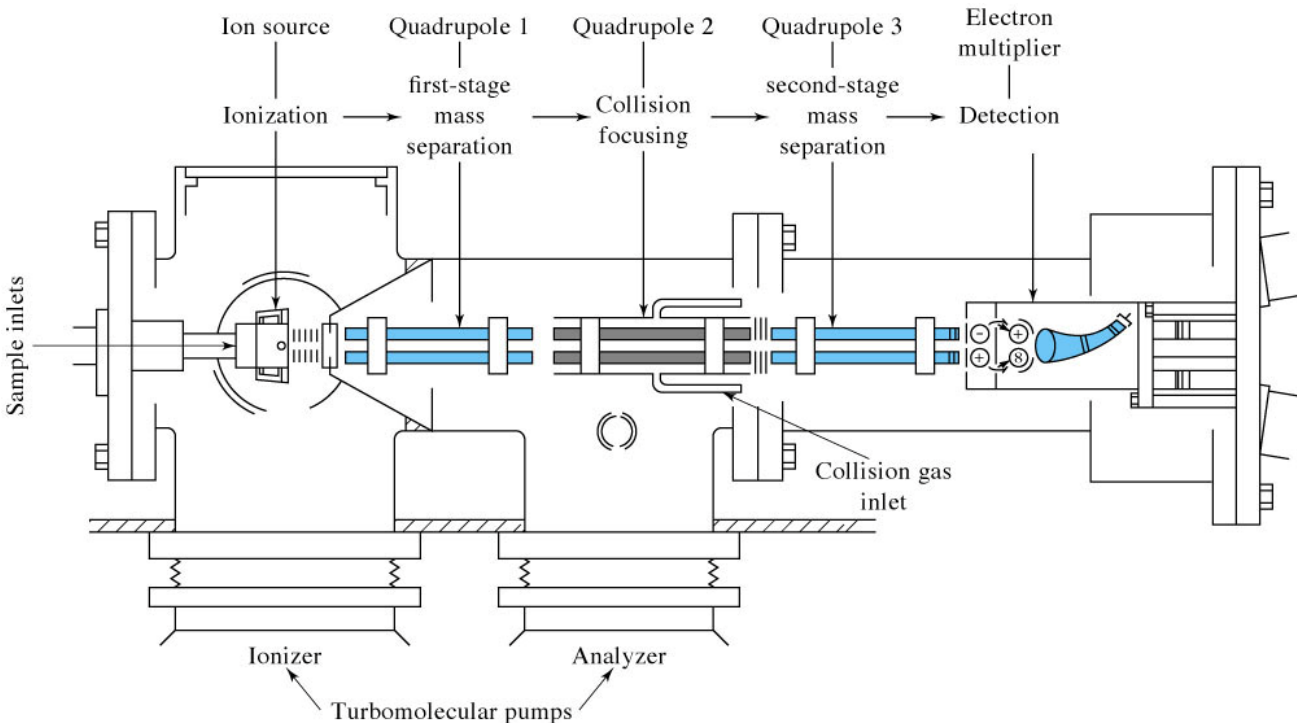


Fig. 20-23

A triple quadrupole mass spec!

A few other helpful videos

- LC-interfaced triple quad MS/MS
  - http://youtu.be/-iLtW6XQMmw

- LC-interfaced qTOF
  - http://youtu.be/BFuZaIi-zDk

Common MS Configurations and Some Applications.

ionization method	*m/z* analyzer	an example application	attainable resolving power [a]	notes
MALDI	TOF	protein identification by database retrieval	10^4	peptide mapping retrieves >70% of proteins[c]
ESI	quadrupole	parent ion scanning (PIS)	10^3	triple quadrupole required for PIS and MS/MS
ESI	ion trap	miniaturized ESI and MS/MS for database retrieval	10^4	lower cost; retrieval often more reliable
ESI	quadrupole-Time-of-Flight (hybrid)	non-covalent interactions	10^4	high *m/z* range (>20,000 *m/z*)
ESI	FTMS	Electron Capture Dissociation	10^6	MS/MS above 10 kDa

[a] Resolving power is $m/\Delta m$ (analyte mass / resolvable mass difference).

Neil L. Kelleher, "From Primary Structure to Function: Biological Insights from Large Molecule Mass Spectra," *Chem. & Biol.*, 2000, *7*, R37-R45.

Applications of molecular mass spectrometry

- Versatile detection technology
  - Not only detects, but provides additional detail in the detection process.
- Qualitative and quantitative applications
  - Identification of compounds and determination of purity
  - Hyphenated techniques
    - GC/MS, LC/MS, CE/MS, GC/MS/MS, LC/MS/MS, and LC/MS^n

TABLE 20-5 Applications of Molecular Mass Spectrometry

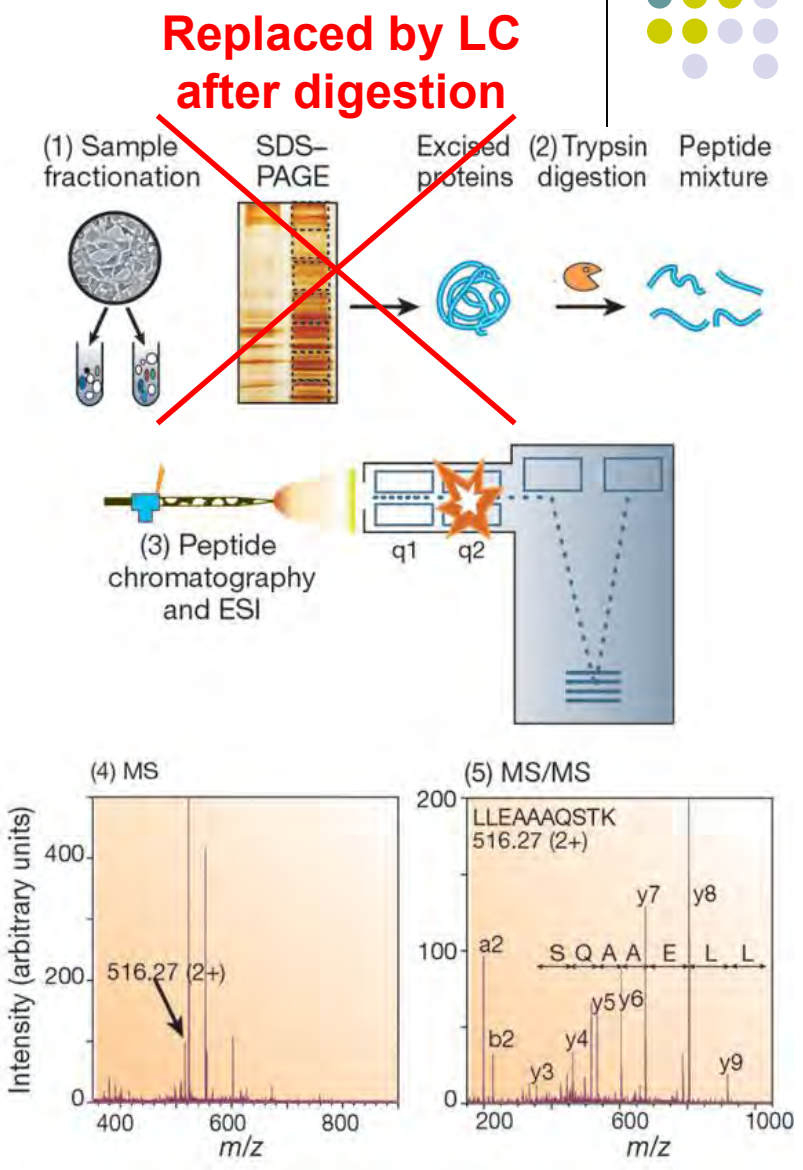
1. Elucidation of the structure of organic and biological molecules
2. Determination of the molecular mass of peptides, proteins, and oligonucleotides
3. Identification of components in thin-layer and paper chromatograms
4. Determination of amino acid sequences in sample of polypeptides and proteins
5. Detection and identification of species separated by chromatography and capillary electrophoresis
6. Identification of drugs of abuse and metabolites of drugs of abuse in blood, urine, and saliva
7. Monitoring gases in patient's breath during surgery
8. Testing for the presence of drugs in blood in race horses and in Olympic athletes
9. Dating archaeological specimens
10. Analyses of aerosol particles
11. Determination of pesticide residues in food
12. Monitoring volatile organic species in water supplies

Proteomics also includes concentration determination (expression level profiling)

Traditional MS/MS-based proteomics

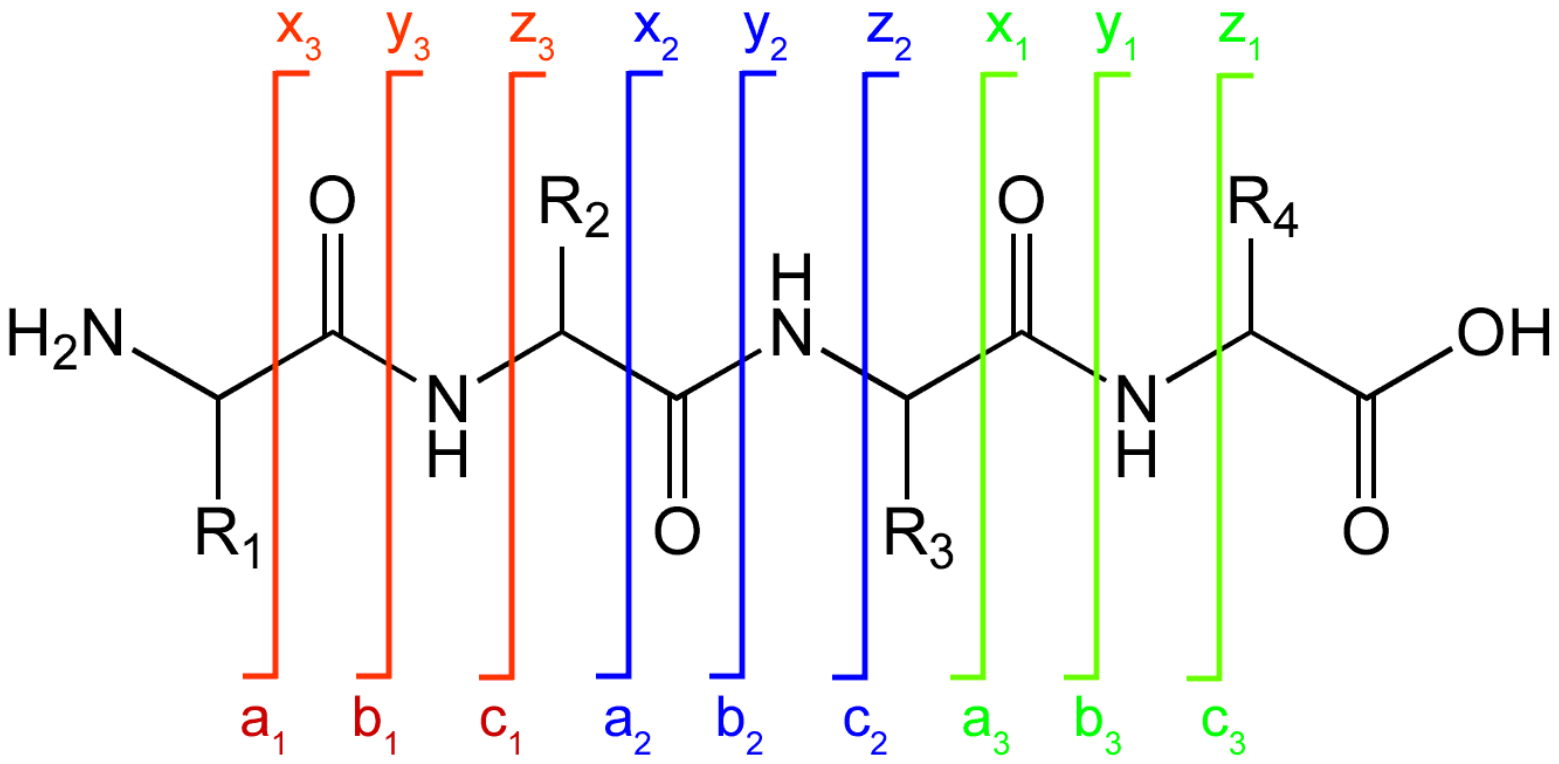
- Proteomics is the large-scale study of proteins, their structure and function

1) Sample collection and 1-D PAGE for rough separation
2) Tryptic digest to yield peptides (more manageable). Whole proteins cannot be unequivocally identified by mass alone.
3) LC purification and ESI injection
4) MS spectrum obtained and peptide composition can be identified, but not sequenced
5) MS/MS fragment analysis allows the linear sequence to be reconstructed



Nature, **2003**, *422*, 198-207.

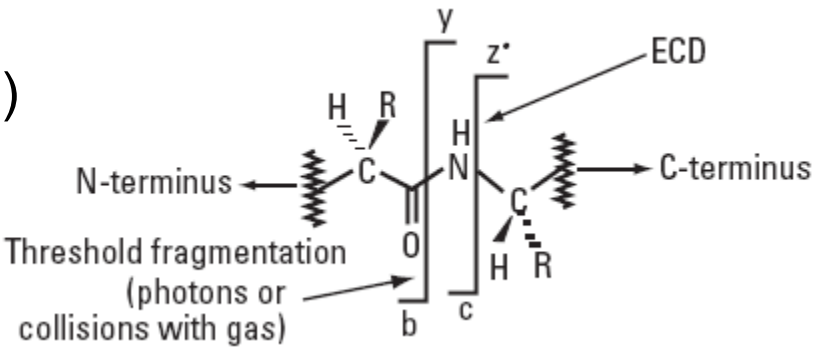
Types of peptide fragments



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

Common non-proteolytic fragmentation methods

- Collision activated dissociation (CAD)
  - Collision-induced dissociation (CID)
  - Fragments at amide bonds giving b and y ions
- Infrared multiphoton dissociation (IRMPD)
  - Fragments at amide bonds giving b and y ions
- Electron capture dissociation (ECD)
  - Low energy electrons used to fragment peptide often at backbone giving c and z ions
  - Mainly used on FT- instruments
- Electron transfer dissociation (ETD)
  - Radical anions (antracene and azobenzene) used to fragment molecular ions (not direct fragmentation) giving c and z ions
  - Also applicable to simpler quadrupole instruments



Both are very useful for studying post-translational protein modifications because they only cleave the backbone of the polypeptide

Proteomics: Protein and peptide sequencing using MS/MS

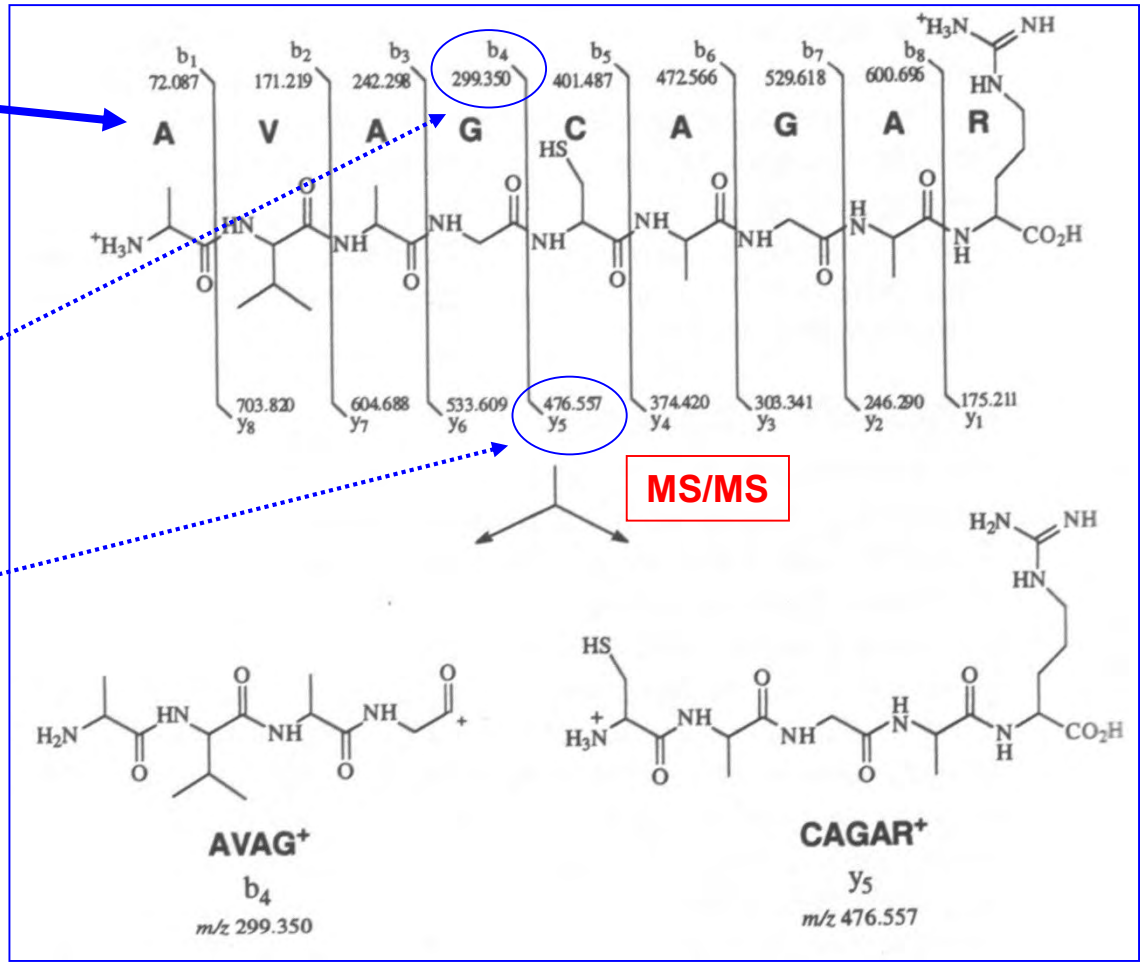
Single-letter amino acid designations

b_n is the left fragment

y_m is the right fragment

$b_n + y_m = 775 = MW$

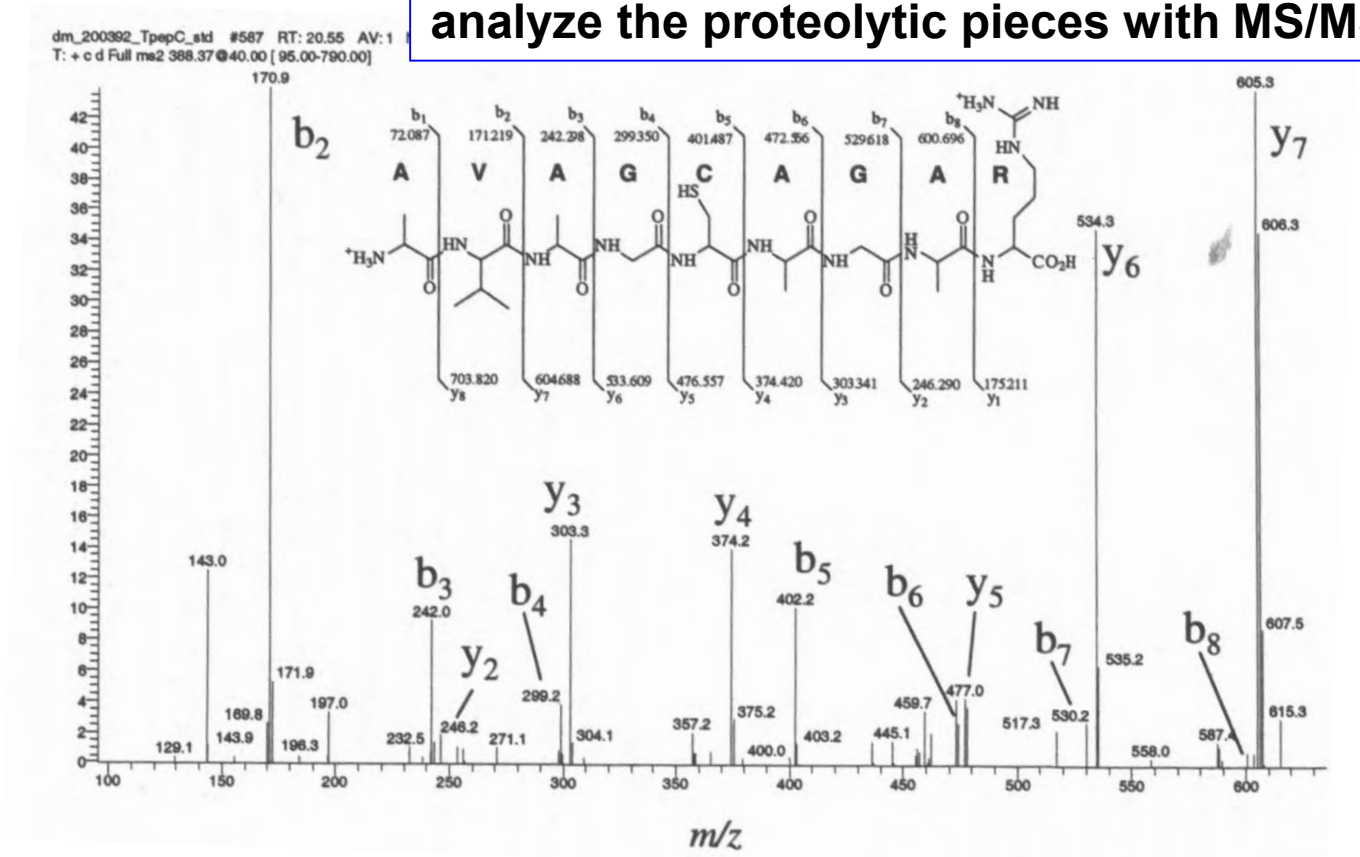
Fragments obtained by proteolytic digestion



From *Introduction to Proteomics: Tools for the New Biology*, by Daniel C. Liebler, 2002, Humana Press

Proteomics: Protein and peptide sequencing using MS/MS

The "bottom-up" approach to analysis: analyze the proteolytic pieces with MS/MS



Problem: Often only partial peptide sequence coverage is achieved

From *Introduction to Proteomics: Tools for the New Biology*, by Daniel C. Liebler, 2002, Humana Press