

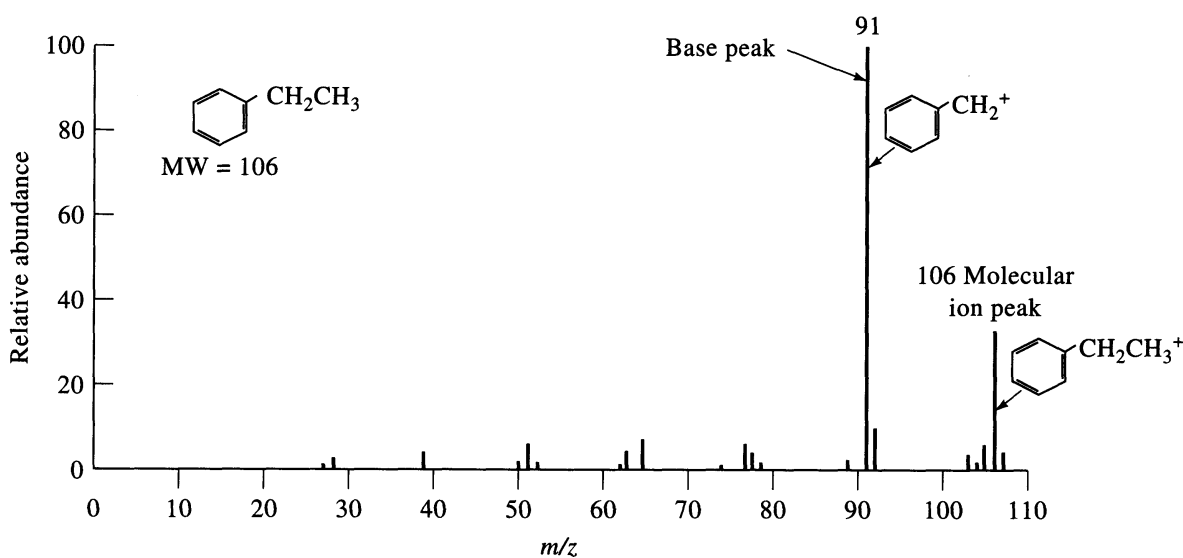
## Mass Spectrometry (Chapter 20):

Based on **ionization** of gas phase molecule followed by analysis of the **masses** of the ions produced

The Mass Spectrum:

Graph of **ion intensity** versus **mass-to-charge ratio** ( $m/z$ ) (units daltons, Da)

Fig 20-1



*molecular ion peak* ( $M^+$ )  $m/z$  corresponds to MW of singly-charged molecule

*fragment peak*  $m/z$  less than MW of singly-charged molecule

*base peak* most intense  $m/z$

## Instrument Components:

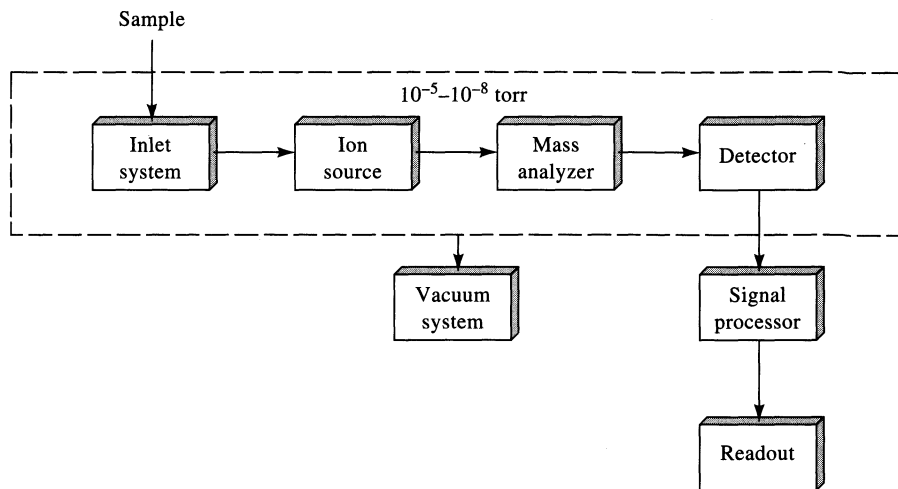


Fig 20-10

- sample introduction system - **vaporize** sample
- ion source - **ionizes** analyte gas molecules
- mass analyzer - **separates** ions according to  $m/z$
- detector - **counts** ions
- vacuum system - **reduces collisions** between ions and gas molecules

## Ion sources:

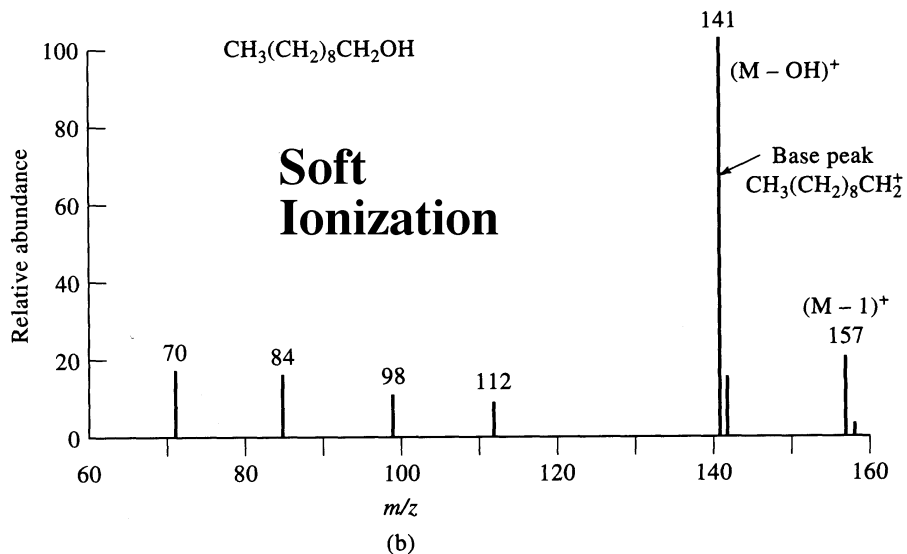
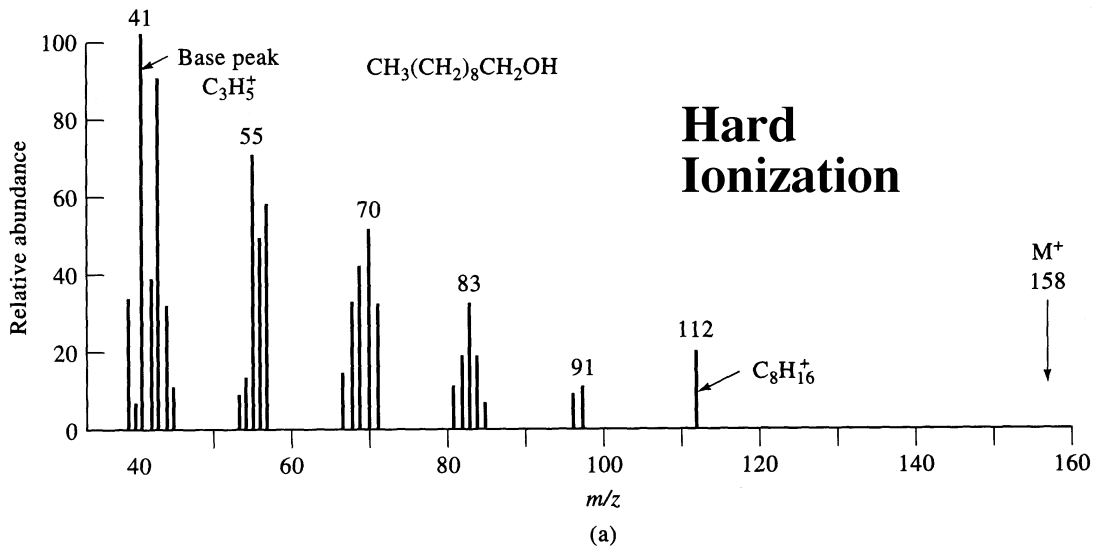
**TABLE 20-1 Ion Sources for Molecular Mass Spectrometry**

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 $^{252}\text{Cf}$
	Fast atom bombardment (FAB)	Energetic atomic beam
	Secondary ion mass spectrometry (SIMS)	Energetic beam of ions
	Thermospray ionization (TS)	High temperature

**Hard** ion sources leave excess energy in molecule - **extensive fragmentation**

**Soft** ion sources little excess energy in molecule - **reduced fragmentation**

Fig 20-2



## Gas Phase Ion Sources:

### (A) **Electron Impact (EI) Ion Source:**

Electron bombardment of gas/vapor molecules

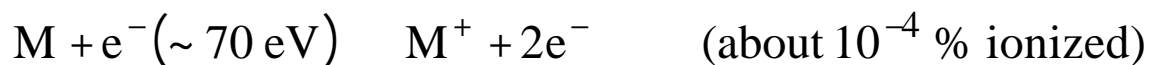
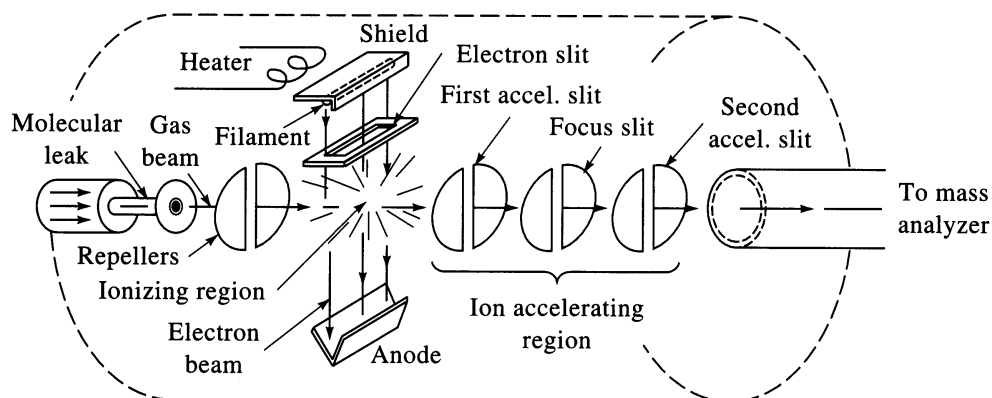


Fig 20-3



Electron energy  $\sim 70 \text{ eV}$

$$\begin{aligned} 1 \text{ eV} &= 1.6 \times 10^{-19} \text{ C} \times 1 \text{ V} && (1 \text{ V} = 1 \text{ J C}^{-1}) \\ &= 1.6 \times 10^{-19} \text{ J} \\ &= 96.486 \text{ kJ mol}^{-1} \end{aligned}$$

## EI Spectra:

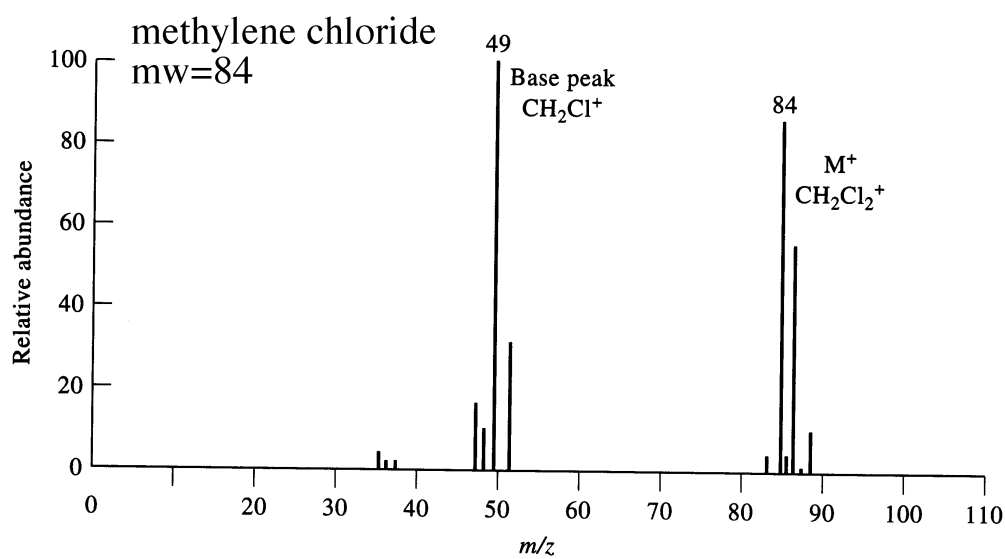
- **hard** source (incident energy 70 eV » than chemical bond)
- molecules electronically, vibrationally and rotationally excited
- extensive **fragmentation** fragment ions

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

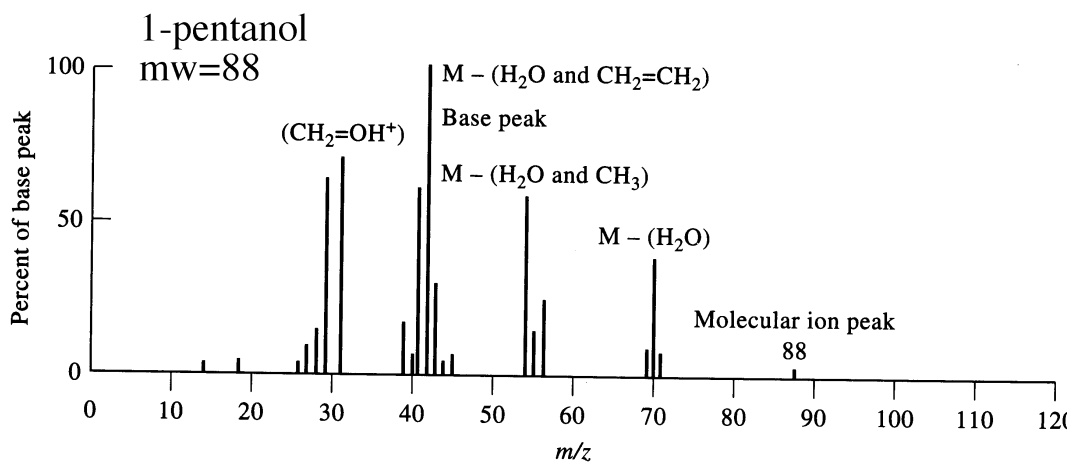
Molecular ion formation	$ABCD + e^- \rightarrow ABCD^{*+} + 2e^-$	
Fragmentation	$ABCD^{*+} \rightarrow A^+ + BCD^*$	
	$\begin{array}{l} \rightarrow A^* + BCD^+ \rightarrow BC^+ + D \\ \rightarrow CD^* + AB^+ \rightarrow \begin{cases} B + A^+ \\ A + B^+ \end{cases} \\ \rightarrow AB^* + CD^+ \rightarrow \begin{cases} D + C^+ \\ C + D^+ \end{cases} \end{array}$	
	Rearrangement followed by fragmentation	$ABCD^{*+} \rightarrow ADBC^{*+} \rightarrow \begin{cases} BC^* + AD^+ \\ AD^* + BC^+ \end{cases}$
	Collision followed by fragmentation	$ABCD^{*+} + ABCD \rightarrow (ABCD)^{*2+} \rightarrow BCD^* + ABCDA^+$

- base peak  $m/z \ll M^+$
- **complex spectra**
  - **helps identification**
  - **poor for measuring MW** of compound

# Fragmentation patterns (Fig 20-4):



(a)

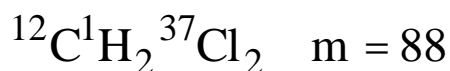
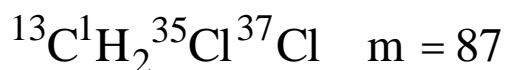
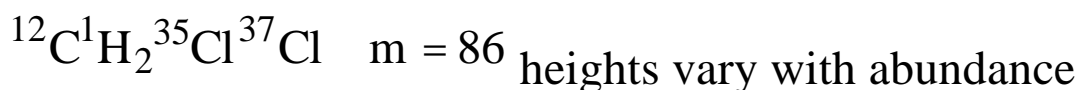
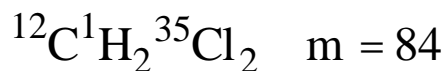


(b)

What about peaks at greater m/z than M+?

Two sources:

- **Isotope Peaks** - same chemical formula but different masses



$^{13}\text{C}$  is 1.1 %  $^{12}\text{C}$ ,  $^{37}\text{Cl}$  is 32.5 %  $^{35}\text{Cl}$

- **Collision Product Peaks** - only common peak is proton transfer to give (M+1)<sup>+</sup> peak (increases with increasing pressure)

Advantages of EI:

- high ion currents - **sensitive**
- fragmentation aids **identification**

Disadvantages of EI:

- weak or absent M<sup>+</sup> peak inhibits **determination of MW**
- molecules must be **vaporized** (MW < 10<sup>3</sup> Da)
- molecules must be **thermally stable** during vaporization



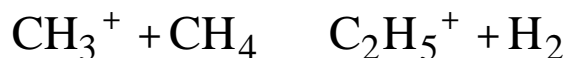
## (B) Chemical Ionization:

- Many modern MS instruments can perform chemical ionization in addition to EI

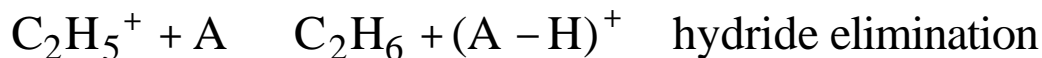
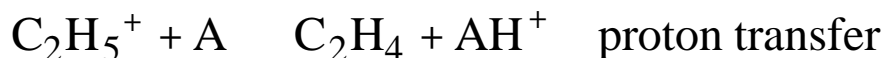
EI ionization in excess (analyte 10-100 ppm) of **reactant gas**

Most common reactant gas is **methane**

EI ionization of methane produces



These **ions react with analyte**:



analyte

- most common ions **(M+1)<sup>+</sup>** and **(M-1)<sup>+</sup>**
- sometimes **(M+17)<sup>+</sup>** (addition of CH<sub>5</sub><sup>+</sup>) or **(M+29)<sup>+</sup>** (addition of C<sub>2</sub>H<sub>5</sub><sup>+</sup>)

## Desorption/Ionization Sources:

Applicable to non-volatile ( $>10^5$  Da) or non-stable analytes

Energy applied to analyte causing desorption and ionization

Exact mechanisms still under investigation

### (A) **Electrospray Ionization (ESI):**

- Explosion of charged droplets containing analytes
  - solution analyte pumped through **charged** (1-5 kV) **capillary**
  - small **droplets** become charged
  - **solvent evaporates**, drop shrinks, surface charge density increases
  - charge density reduced by expulsion of charged analyte molecules ("**Coulomb explosion**")

Soft ionization - **little fragmentation**

Easily adapted to FIA, capillary EP and HPLC

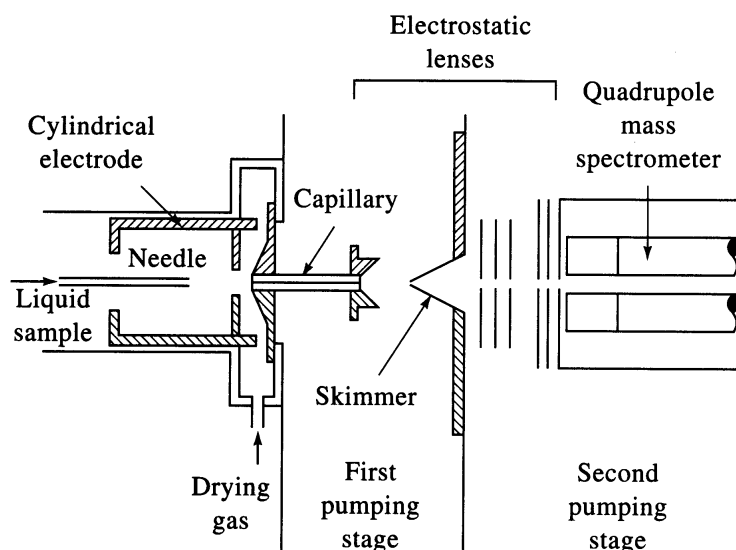


Fig 20-8

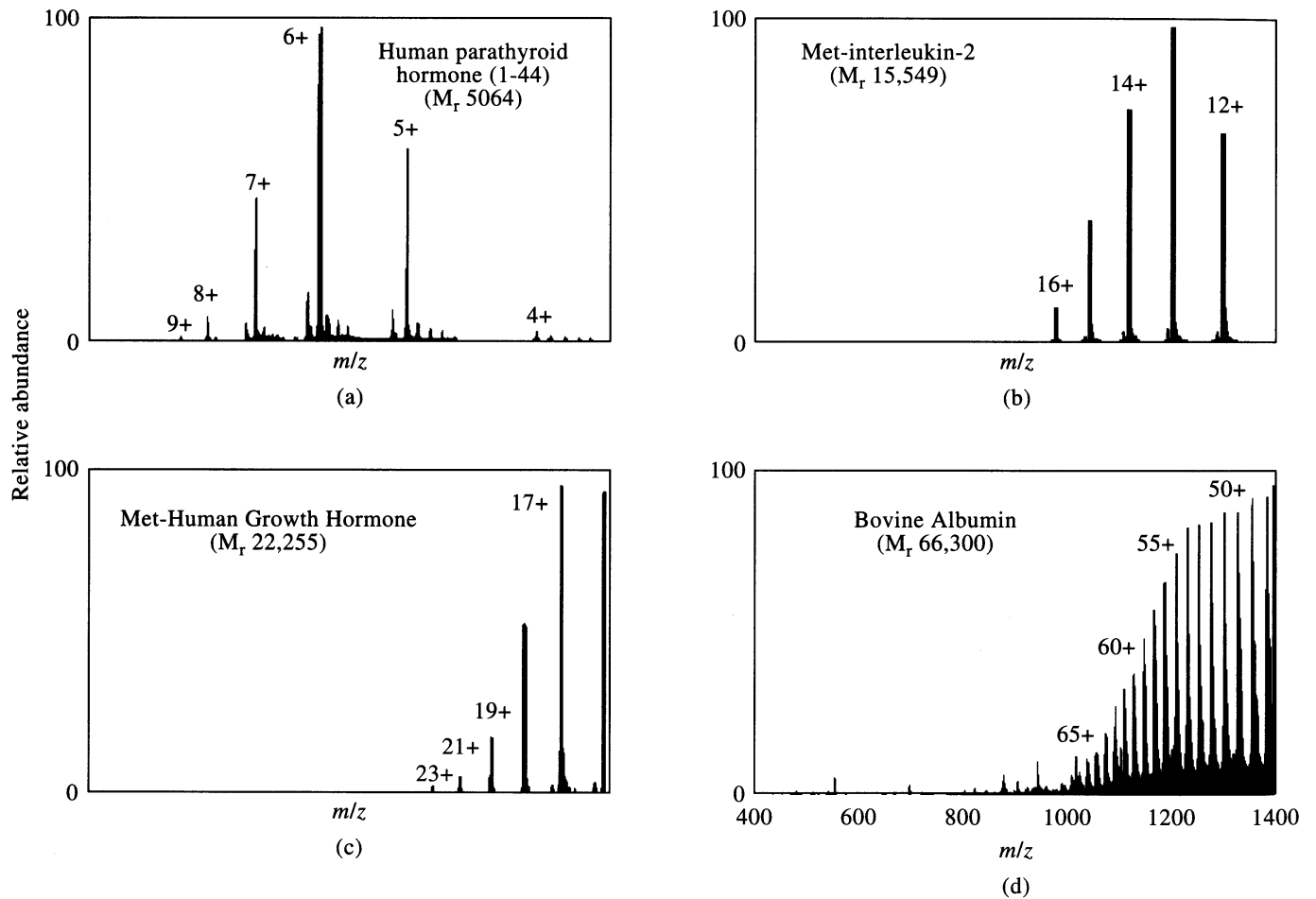
Very **important technique** for **large** ( $>10^5$  Da) thermally **fragile** molecules

- peptides
- proteins
- polymers
- oligonucleotides

Analytes may accumulate **multiple charges** in ESI

- $M^+$ ,  $M^{2+}$ ,  $M^{3+}$ ...

Fig 20-9



(B) **Fast Atom Bombardment (FAB):**

Hard ionization - fragmentation

Sample in **glycerol** solution

Bombarded by high energy **Ar or Xe atoms** (few keV)

Atoms and ions **sputtered** from surface (ballistic collision)

Both **M<sup>+</sup>** and **M<sup>-</sup>** produced

Applicable to small or large (>10<sup>5</sup> Da) unstable molecules

(C) **Matrix-Assisted Laser Desorption/Ionization (MALDI):**

Soft ionization

- analyte dissolved in **solution of UV-absorber** and solvent
- solid **crystals** of analyte + absorber grow (matrix)
- **pulsed laser** fired at crystals in time-of-flight mass spectrometer (TOF-MS)
- **molecular ion desorbed** from crystal surface

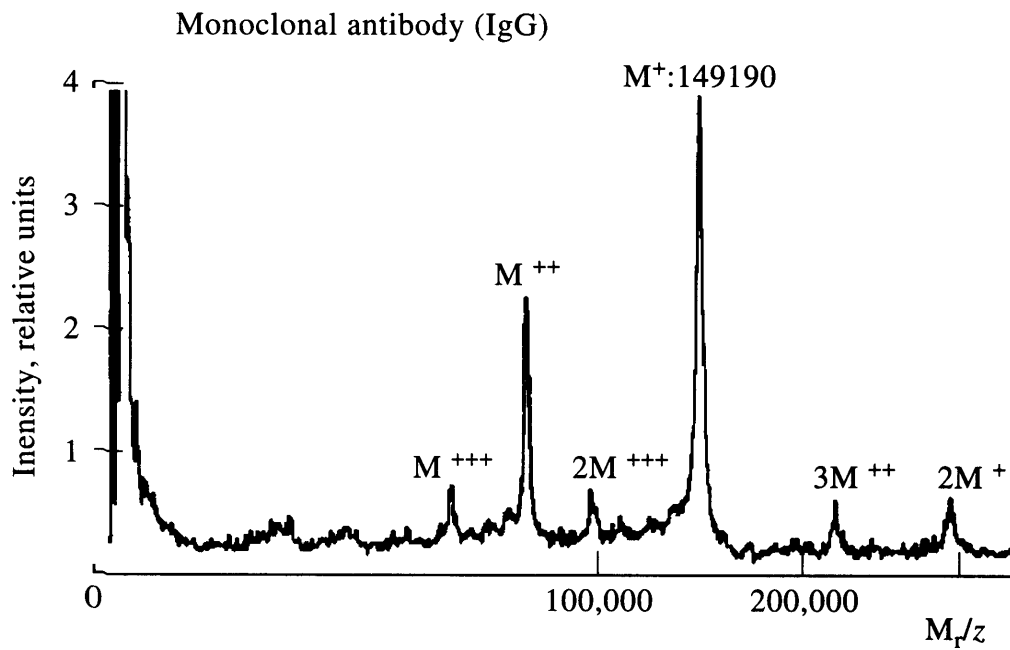
MALDI spectrum contains

dimer, trimers...

multiply charged molecules

no fragmentation

Fig 20-7



**TABLE 20-4** Matrices Most Frequently Used for MALDI Together with the Usable Wavelengths\*

Matrix	Wavelength (nm)
Nicotinic acid	266, 220–290
Benzoic acid derivatives:	
2,5-Dihydroxybenzoic acid	266, 337, 355
Vanillic acid	266
2-Amino-benzoic acid	266, 337, 355
Pyrazine-carboxylic acid	266
3-Aminopyrazine-2-carboxylic acid	337
Cinnamic acid derivatives:	
Ferulic acid	266, 377, 355
Sinapinic acid	266, 337, 355
Caffeic acid	266, 337, 355
3-Nitrobenzylalcohol	266

\*From M. Karas and U. Bahr, *Trends Anal. Chem.*, 1990, 9, 322.

Matrix properties:

small MW

absorb UV

able to crystallize

## Mass Analyzers:

Mass analyzers: **separate** ions to **measure** m/z and intensity

## Resolution:

- ability to differentiate peaks of similar mass

$$R = \frac{\text{mean mass two peaks}}{\text{separation between peaks}} = \frac{\bar{m}}{m}$$

- resolution **depends on mass!**

- if  $R = 1000$

separate peaks at  $m/z = 100$  and  $100.1$

or  $m/z = 1000$  and  $1001$

or  $m/z = 10000$  and  $10,000$

- high resolution necessary for exact MW determination:

nominal MW = 28

actual MW  $C_2H_4^+ = 28.0313$

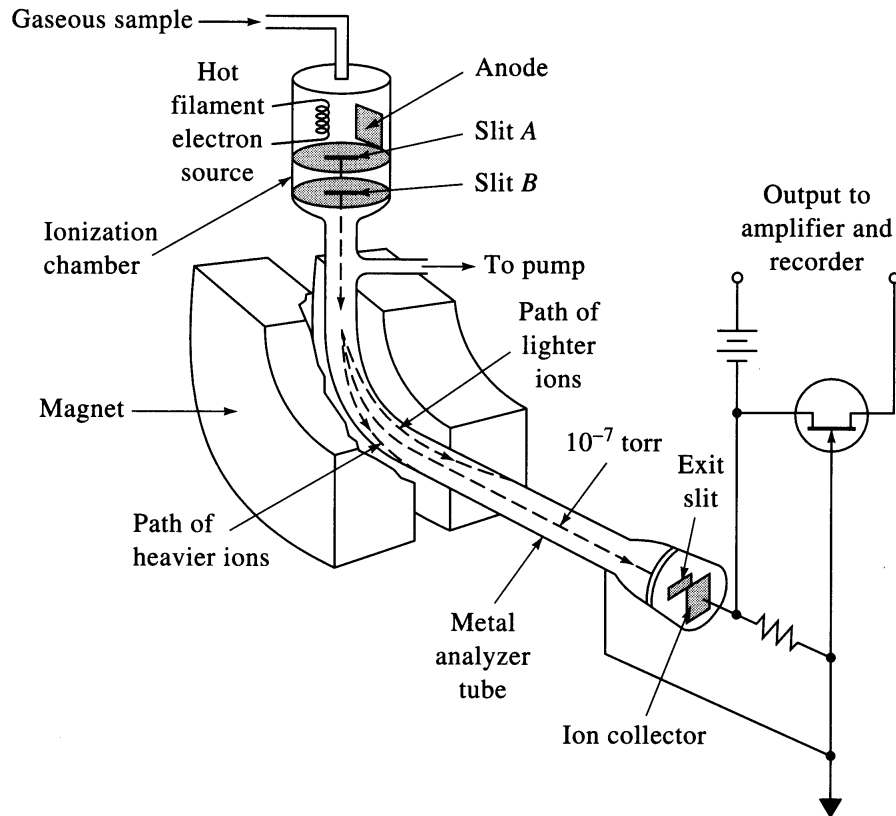
$CH_2N^+ = 28.017$

$N_2^+ = 28.0061$

$R > 2570$

(A) Magnetic Sector Analyzers:

Fig 20-12



**Kinetic energy** of ion:

$$KE = z e V = \frac{1}{2} m v^2$$

charge (+1, +2...)

velocity (m/s)

electronic charge (1.6x10<sup>-19</sup> C)

mass (kg)

potential difference (B-A)



Magnetic force:

$$F_B = B z e v$$

Centripetal force:

$$F_C = \frac{m v^2}{r}$$

radius of sector (m)

For successful exit, two forces **balance**

$$B z e v = \frac{m v^2}{r} \quad v = \frac{B z e r}{m}$$

$$\frac{m}{z} = \frac{B^2 r^2 e}{2V}$$

For fixed radius and charge can

- (a) use permanent magnet, vary A and B potential (V)
- or (b) variable electromagnet, fixed A and B potential (V)

(B) Double-Focusing Analyzers:

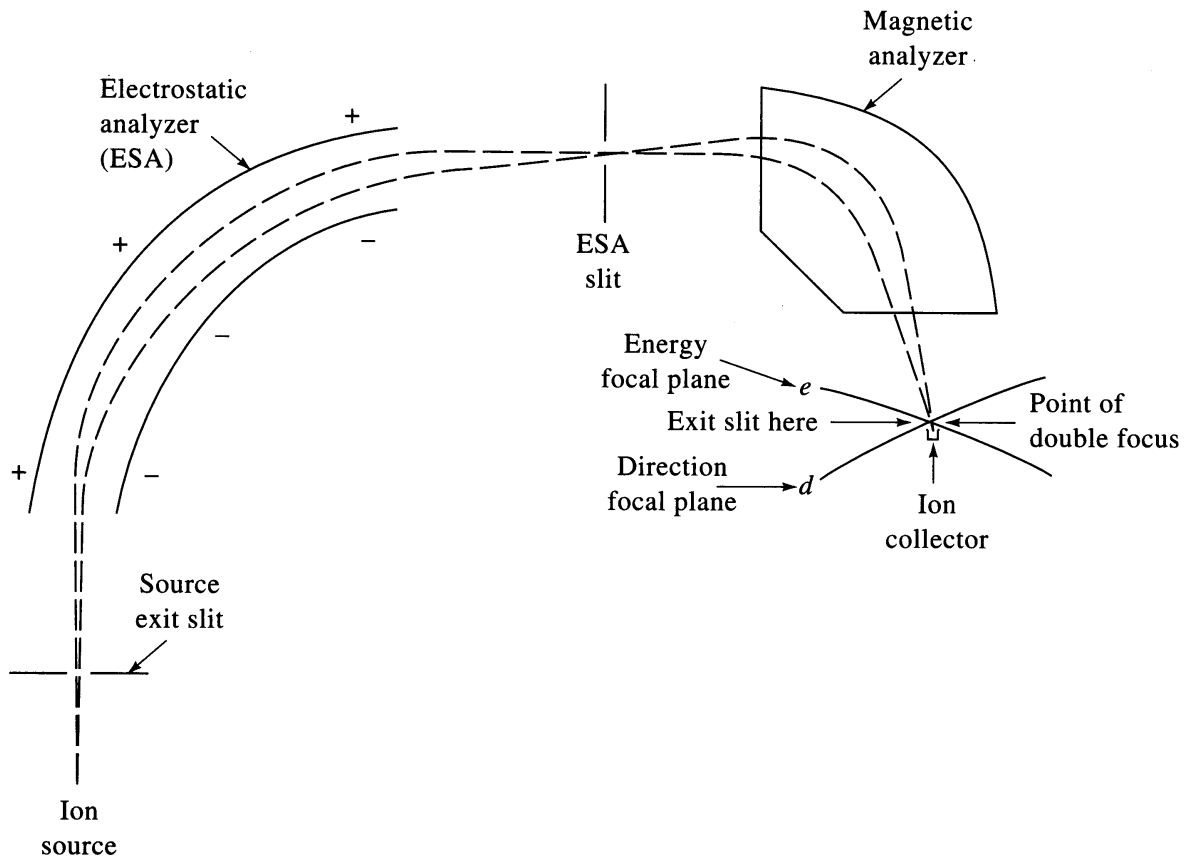
Single-focusing magnetic sector analyzers have  $R_{\max} < 2000$

- (a) translational energy aberrations
- (b) angular aberrations

Addition of electrostatic analyzer simultaneously minimizes both (a)&(b)

Electrostatic analyzer focuses ions of unique  $m/z$  at entrance slit to magnetic sector

Fig 20-13



(C) **Quadrupole Analyzers** (see also 11 B-2):

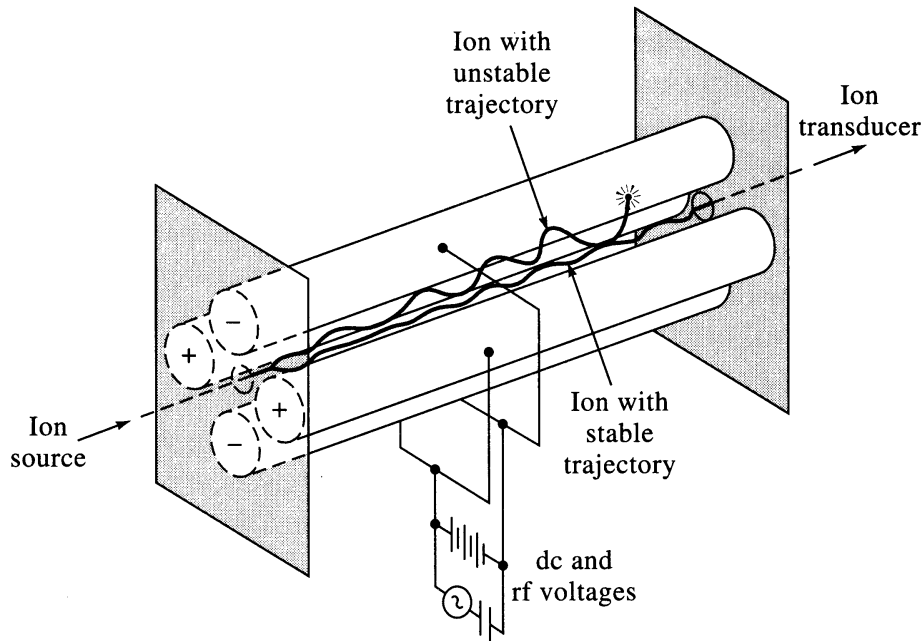


Fig 11-4

- ions travel parallel to four rods
  - opposite pairs of rods have rapidly alternating potentials (AC)
  - ions try to follow alternating field in helical trajectories
  - stable path only for one  $m/z$  value for each field frequency
- 
- Harder to push heavy molecule -  $m/z_{\max} < 2000$
  - $R_{\max} \sim 500$

(D) Time of Flight (TOF) Analyzers:

Generate pulse of ions (by laser, electrons) with same initial energy

Ions travel down field-free tube separate according to mass

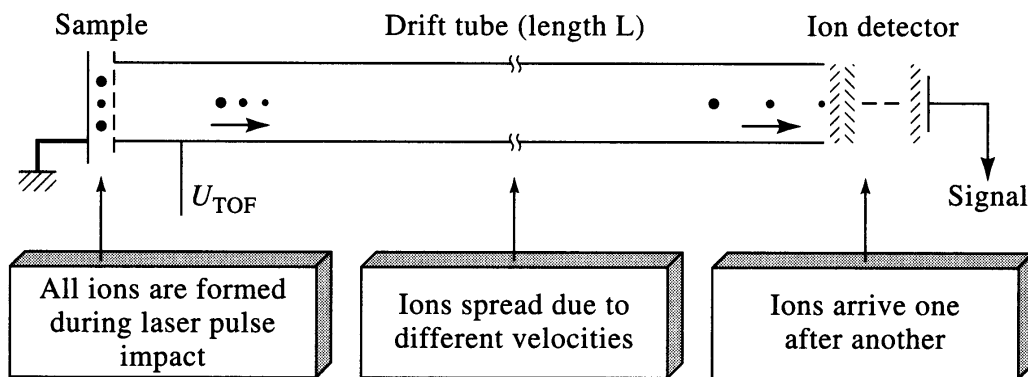
- light ions arrive first, heavy ions arrive later

Unlimited mass range  $m/z_{\max} > 100 \text{ kDa}$

Poor resolution  $R_{\max} < 1000$

Poor sensitivity

Fig 11-8

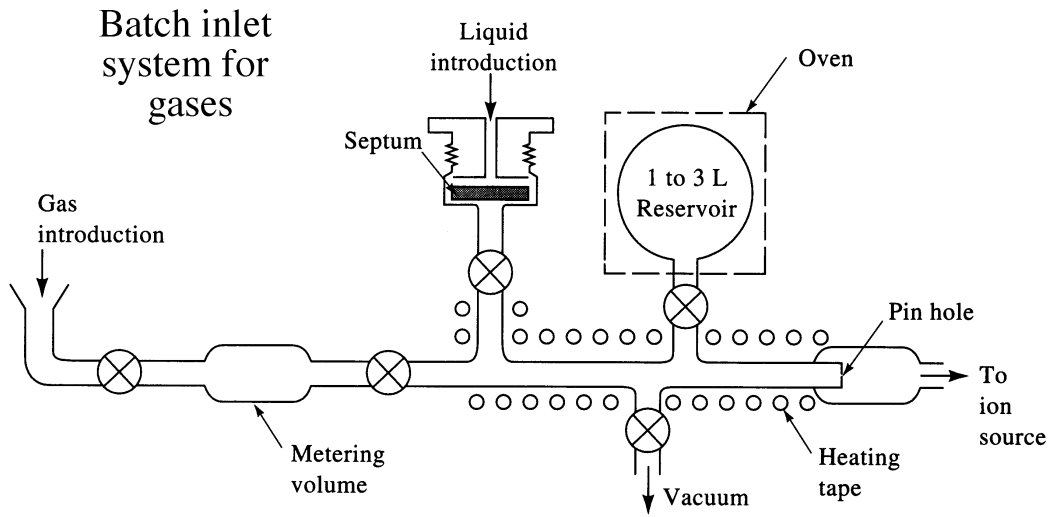


$$\text{KE} = \frac{1}{2} mv^2 = \frac{1}{2} m \frac{L}{t}^2$$

$$t = \sqrt{\frac{1}{2} mL^2} \quad m \quad t^2$$

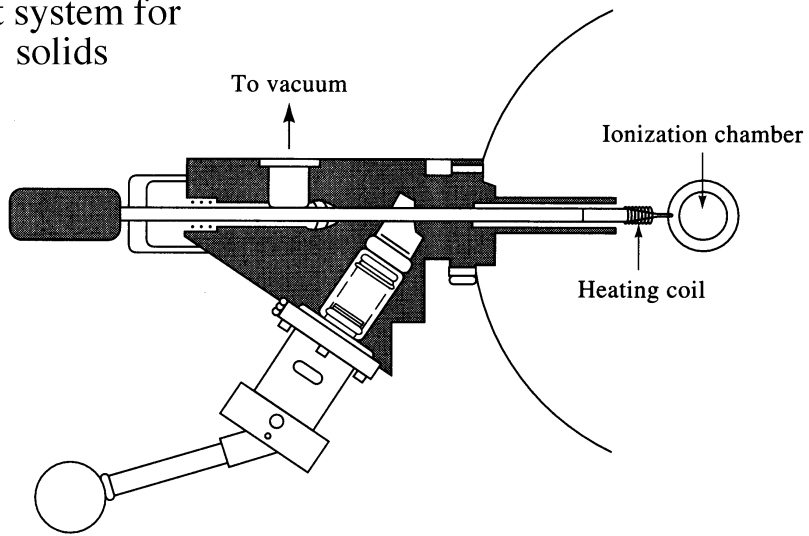
# Sample Introduction:

Fig 20-11



(a)

## Direct probe inlet system for solids



(b)

(A) **External (Batch) Inlet Systems:**

Sample heated (<400 °C) in small external oven

Vapor admitted to ionizer through valve

Gas stream added to entrain analyte

(B) **Direct Probe**

Sample vial inserted through air-lock into ionizer chamber

Vial heated to vaporize sample

Vial can be reduced to capillary or surface plate for small quantities

(C) **Chromatography/Electrophoresis/Injection Analysis**

Can be modified to directly flow into ionizer region

## Applications of Molecular MS

### Identification of Pure Compounds:

- (a) **Nominal M<sup>+</sup> peak** (one m/z resolution) (or (M+1)<sup>+</sup> or (M-1)<sup>+</sup>) gives **MW** (not EI)
- (b) **Exact m/z** (fractional m/z resolution) can give **stoichiometry** but **not structure** (double-focusing instrument)
- (c) **Fragment** peaks give evidence for **functional groups**
- (M-15)<sup>+</sup> peak methyl
  - (M-18)<sup>+</sup> OH or water
  - (M-45)<sup>+</sup> CO<sub>2</sub>H
  - series (M-14)<sup>+</sup>, (M-28)<sup>+</sup>, (M-42)<sup>+</sup>... sequential CH<sub>2</sub> loss in alkanes
- (d) **Isotopic peaks** can indicate presence of certain **atoms**
- Cl, Br, S, Si
- (e) **Isotopic ratios** can suggest plausible molecules from M<sup>+</sup>, (M+1)<sup>+</sup> and (M+2)<sup>+</sup> peaks
- $^{13}\text{C}/^{12}\text{C} = 1.08\%$   $^2\text{H}/^1\text{H} = 0.015\%$
- (M+1)<sup>+</sup> peak for ethane C<sub>2</sub>H<sub>6</sub> should be  
(2x1.08)+(6x0.015) = 2.25% M<sup>+</sup> peak
- (f) Comparison with **library spectra**

## Summary of MS

One of most powerful analytical tools:

- **sensitive** ( $10^{-6}$  to  $<10^{-13}$  g)
- **range of ion sources** for different situations
- **elemental composition** for small and large MW
  - biomolecules
- limited structural information
- **qualitative** and **quantitative** analysis of mixtures
- composition of solid surfaces
- isotopic information in compounds

But:

- **complex** instrumentation
- **expensive**
  - high resolution
- structure obtained indirectly
- **complex spectra/fragmentation** for hard ionization sources
- simple spectra for soft ionization sources