

# Mass Spectrometry

Chem 221  
Instrumental Analysis  
Spring 2005

## Background

- Method for *separating* and *quantifying gas phase ions* according to their **mass-to-charge ratio**
- NOT A SPECTROSCOPIC METHOD!**  
-no EMR interactions measured
- Mass Spec is a SEPARATIONS technique**  
-ions are *separated physically* based on mass

2

## General Instrumental Scheme

### Ion Source:

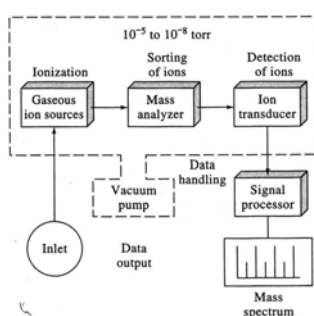
- converts sample to gas phase
- ionizes sample

### Mass Analyzer:

- "sorts" ions according to mass-to-charge ratio

### Detector:

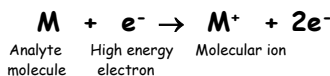
- converts ions to electrical signal proportional to # of ions



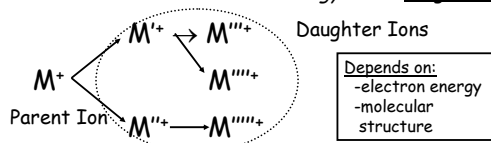
## How are ions generated?

- Simplest, most common method:

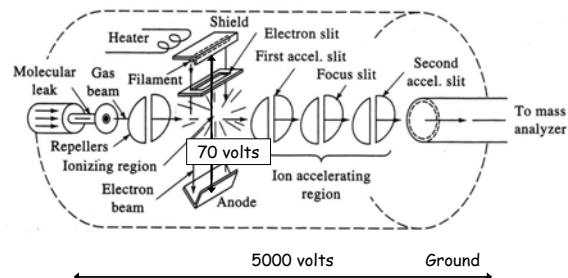
### *Electron Impact (EI) Ionization*



- if  $M^+$  has sufficient *internal energy* it can **fragment**:



## Electron Impact Ion Source



5

## Electron Impact Ionization

- Poor Ionization Efficiency**  
-only about  $10^{-4}$  % ionized
- Variable Electron Gun Potential**  
-adjustable from 0 to 70 volts  
-most covalent molecules ionized at 10 eV  
-excess energy results in *fragmentation*

-many other types of ionization sources!

6

## How are Ions Detected?

- **Electron Multiplier** - fast, sensitive



-like a PMT for ions

**Also:** Faraday Cup, photoplates, etc.

7

## How are ions separated?

- **Three Types of Mass Analyzers:**

1. **Magnetic Sector**  
-physical separation
2. **Time-of-Flight**  
-temporal separation
3. **Quadrupole**  
-"trajectory stability" separation

All require low pressure operation.

8

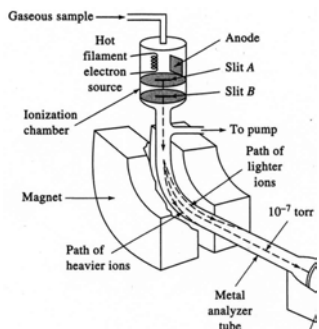
## Magnetic Sector Analyzers

•Ions moving in magnetic field will have a force exerted on them

•Radius of curvature will vary with  $m/z$ :

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

Where: B = magfield  
V = accel. potential  
r = radius of sector



## More MagSector Analyzers

- "Scan" by varying *either* magfield (B) or acceleration potential (V)

➢ Only ions having a  $m/z$  giving correct  $r$  will reach exit slit

- **NOTE:**  $r \propto (m/z)^{1/2}$   
-so, as  $m/z$  increases, the change in  $r$  will decrease
- Result:** ion separation gets poorer as  $m/z$  increases

10

## Properties of MagSectors

- **Limited Resolution**  
-resolution controlled by *spread of K.E.* of ions from the ion source

- **Quantifying Resolution**

$$R = m/\Delta m$$

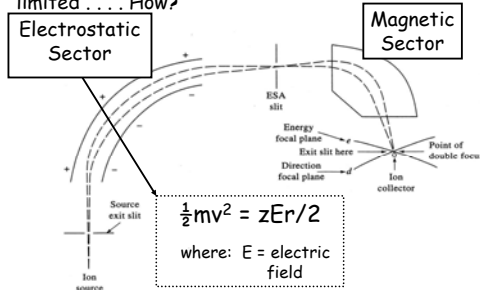
-for magsector: R is typically ~500-1000

**Example:**  $N_2^+$  (28.00616 amu) versus  $CO^+$  (27.99491 amu)

$R = 28/0.01125 \approx 2500$  (magsector not good enough)

## Double-Focusing Analyzers

- Resolution can be improved if the K.E. of the ions is limited . . . How?



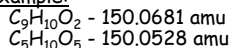
12

## Properties of Double-Focusing Analyzers

### Resolution can be improved dramatically

- $R \approx 20,000 - 50,000$  is common
- $R = 150,000$  is possible
- *Best resolution* of the 3 mass analyzers we are covering

### Example:



-need analyzer with  $R$  of  $\sim 10,000$

13

## Time-of-Flight (TOF) Mass Analyzers

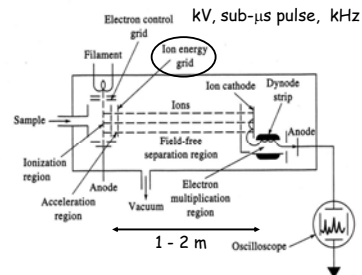
### Simple concept:

- ✓ Accelerate all ions to same K.E.
- ✓ Velocities of ions will vary with their masses:

$$v = (2eV/m)^{\frac{1}{2}}$$

cm/sec      mass/ion (grams)

$1.60 \times 10^{-12}$  erg/V



14

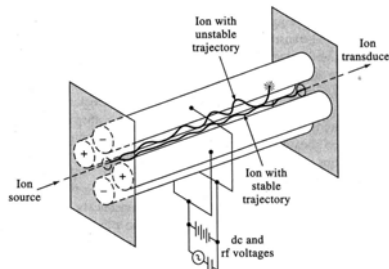
## Properties of TOF Analyzers

- **Resolution:** typically limited to  $\sim 500$
- **FAST!**
  - Some typical transit times:
    - $H^+ \rightarrow 1.58 \mu s$
    - $N_2^+ \rightarrow 8.37 \mu s$
    - $Xe^+ \rightarrow 18.17 \mu s$
- Simple, rugged, smaller than magsectors
- *Ideal* with pulsed ion sources

15

## Quadrupole Mass Analyzers

- Based on *path stability* of ions in an oscillating electric field:



16

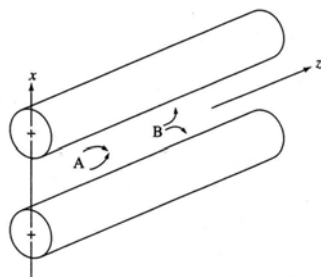
## How do they work?

### DC Potential:

-affects ALL ions (indep of mass)

### AC Potential:

-affects LIGHT ions

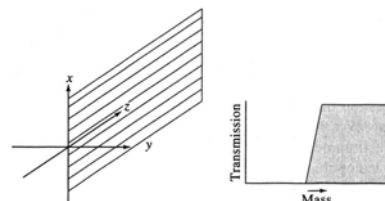


17

## First BiPole Pair: Pos. DC

•• DC potential *repels ALL ions*

• AC (rf) potential *attracts light ions*



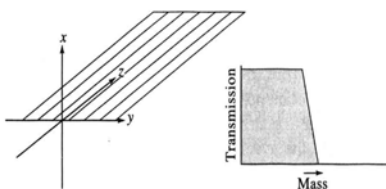
*High Mass Pass Filter*

18

## Second BiPole Pair: Neg. DC

-- DC potential attracts ALL ions

• AC (rf) potential repels light ions

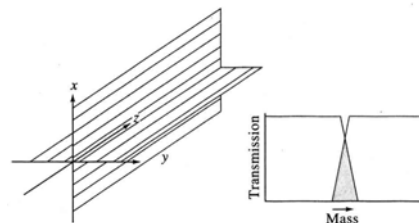


Low Mass Pass Filter

19

## Putting it all together

Combo of the two filters results in transmission of only a narrow range of masses:



• Variable mass filter

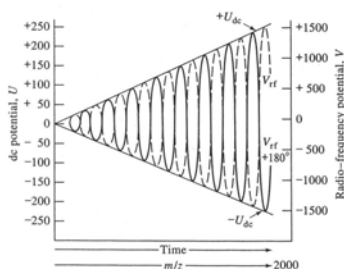
• Mass and mass range vary with ac and dc potentials

20

## Scanning a Quad

### •Resolution:

-controlled by ac/dc (max res. at ~6)

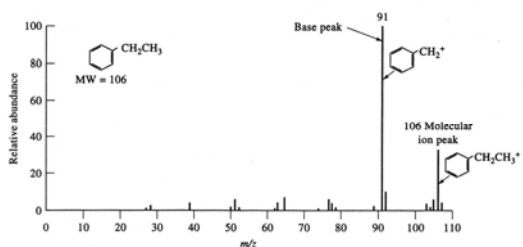


## Properties of Quad Analyzers

- **Resolution:** up to ~1500
- **Fast!** can obtain spectrum in *ms*
- Small, rugged, cheap
- Higher ion throughput than magsectors
  - Circular versus slit aperture

22

## A Mass Spectrum



23

## Applications: MW Determination

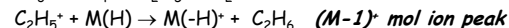
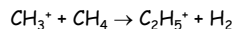
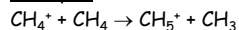
- Need pure compound
- Determine  $m/z$  for molecular ion
  - Vary electron gun potential to confirm molecular ion assignment
  - use a milder ionization method (e.g., Chemical Ionization)

24

## Chemical Ionization (CI)

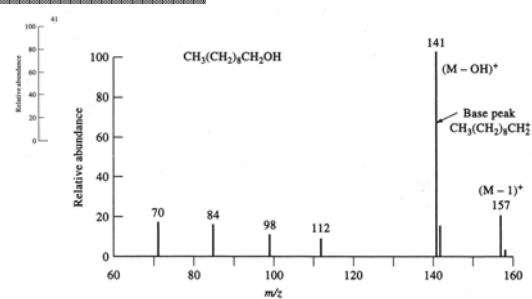
- Add a *reagent gas* to the EI source
- *Reagent ion* is formed by interaction with  $e^-$  beam
- Analyte is ionized by reaction with the *reagent ion*

### ➤ Example:



25

## EI versus CI: Decanol



## Molecular Formula Determination

### ■ **Brute Force Method**

- get molecular weight from mass spectrum
- compare MW with MWs of suspected compounds

BUT, this requires a *high-resolution* mass spectrum . . . not always an available option

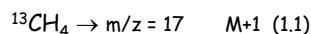
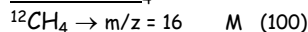
### ■ **Isotope Ratio Method**

- use isotope natural abundances to decipher multiple peaks due to *isotopomers*

27

## Isotope Ratio Method

### ➤ Consider $\text{CH}_4$ :

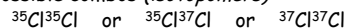


Relative abundance of  $^{13}\text{C} = 1.1\%$  of  $^{12}\text{C}$

### ➤ Consider $\text{Cl}_2$ :

-Relative abundance of  $^{37}\text{Cl} = 33\%$  of  $^{35}\text{Cl}$

-3 possible combos (*isotopomers*):



(56.5%)

(37.3%)

(6.2%)

$M$  (70)

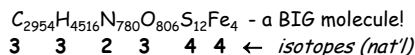
$M+2$  (72)

$M+4$  (74)

28

## More on Isotopes

- Determine abundance *patterns* of molecular ion peak(s) and of fragment peaks
- Patterns are characteristic of:
  - ✓ Element(s) responsible for isotopomers
  - ✓ The *number* of elements in the compound/fragment
- This can get complex . . . consider *hemoglobin*:



(Aside: it is unlikely to find two *identical* hemoglobin molecules in a single drop of blood!)

29

## Molecular Structure Determination

- Fragmentation is due to the *chemistry* of the ions in the ionization chamber and is indicative of *molecular structure*:

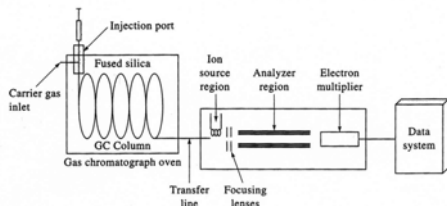
- **Alcohols**: lose water → peak at  $(M-18)^+$
- **Acids**: lose OH → peak at  $(M-17)^+$
- **Amides**: lose  $\text{NH}_2$  → peak at  $(M-16)^+$
- $\text{CH}_3$  is commonly lost → peak at  $(M-15)^+$
- etc!

- Fragmentation pattern can be used as a *molecular fingerprint* for computer library search identification

30

## What about Mixtures?

- Need *pure compounds* for qualitative/structural determinations
  - use an *inline* separation just prior to obtaining mass spectrum: e.g., Gas Chromatography

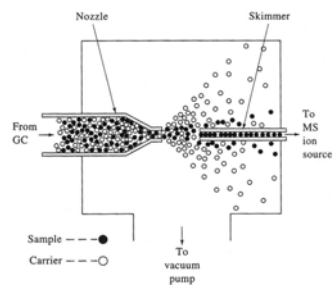


31

## GC-MS: the interface

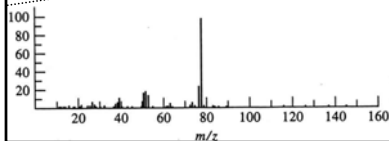
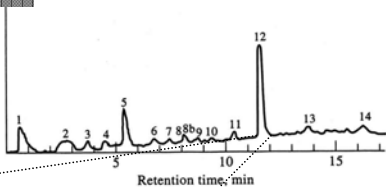
- How do we interface GC effluent (at atmospheric pressure) into reduced pressure Mass Spec?

**The Jet Separator!**



## GC-MS: Dealing with Data Overload

Summed ion current (all  $m/z$ ) versus time gives a **chromatogram**:



Get a mass spectrum for each mixture component

33

## Quantitative Analysis

- Ion current is proportional to # of ions reaching detector, so:

$$I \propto \text{concentration}$$

- Detection Limits:**

depends on ion source

- ~0.1 ppm with EI source
- low-sub ppb possible with proper ion source
- with heavily-fragmented spectra, can *sum ion currents* to increase signal

34

## How is Quantitation Done?

➤ Since:  $I = k \times \text{conc.}$

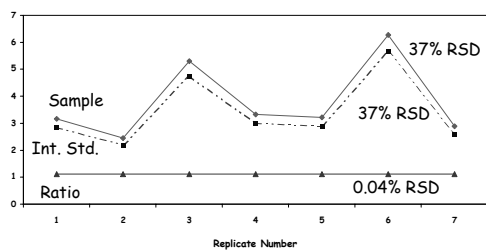
- Determine the value of  $k$  using calibration standard(s)
- Calibration can be difficult due to uncertainties in:
  - Sample introduction
  - Sample ionization

➤ Solution: **Method of Internal Standards**

- Add a *known amount* of *internal standard* to samples and calibration standards
- Use  $I_{\text{analyte}}/I_{\text{IntStd}}$  as figure of merit

35

## Example: Internal Standard



36

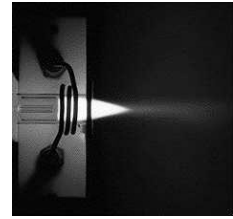
## Mass Spec: The "Ultimate Detector"?

- Provides:
  - Molecular/Atomic weights of analyte
  - Structural details of analyte
  - Sensitive, accurate and precise quantitation
- Easily coupled with many other methods:
  - GC-MS
  - ICP-MS
  - LC-MS
  - Laser-MS
  - MS-MS
  - MS-MS-MS
  - etc.

37

## ICP-MS

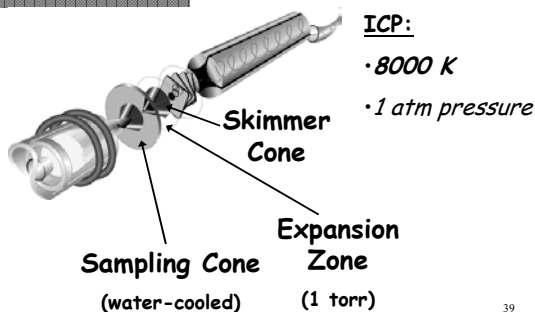
- ICP: great source of atomic ions
  - ✓ many elements
  - >90% ionized at ICP temperatures (8-10,000 K)



*How to interface with MS?*

38

## ICP-MS: Sampling Ions



39

## ICP-MS: Results

- With Quad Mass Analyzer:
  - unit mass resolution
  - detection limits: sub-ppb
  - > 4 decades LDR
  - rapid scanning
  - *isotope ratio* information available
- Also:
  - TOF
  - Double-Focusing (for High-Res) - *why?*

40

## Isotope Dilution MS (IDMS)

- The "Perfect" Internal Standard?
  - Add *known amount of stable isotope* to samples and standards for element of interest
    - ✓ Addition is made *prior* to sample processing (digestion, dilution, etc.)
  - Measure *isotope ratio*
    - ✓ unaffected by physical/chemical changes
    - ✓ unaffected by measurement uncertainties
  - Calculate *sample composition* based on:
    - ✓ known isotope ratio of sample
    - ✓ known isotope ratio and amount of isotope-enriched addition

41

## IDMS - Results?

- Outstanding Accuracy
  - ✓ accounts for errors due to sample processing (loss and contamination) - both physical and chemical
- Outstanding Precision
  - ✓ better than simple internal standards
  - ✓ RSD  $\leq$  0.25%
- *It's the "Definitive Method"*
  - ✓ Used for NIST certification of standard reference materials
  - ✓ Limitations: time, cost, availability of enriched isotopes

42

## LC-MS

### ➤ Trickier than GC-MS (condensed phase):

- convert to vapor phase
- remove solvent
- ionize

### ➤ Strategies

- Moving Belt Interface
- Particle-Beam/Therma-Spray™
- Direct: Electrospray Ionization

43

## Electrospray Ionization (ESI)

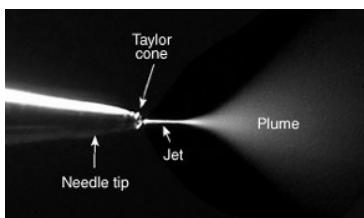
- First modern practical use reported by John Fenn (1985)
  - received Nobel Prize (2002)



44

## ESI - What Is It?

- Introduce sample solution through capillary
- Apply high-voltage (1-8 kV) to capillary
- Get desolvation, charged ions - at atmospheric pressure



45

## ESI - How Does It Work?

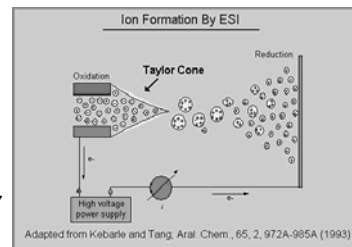
- droplets formed with applied charge

- solvent evaporates:

- ✓ droplet size decreases
- ✓ charge density increases

- "Coulombic Explosion"

- ✓ produces ions with multiple charges



46

## ESI - Interfacing with MS

- ions sent to *heated capillary*
  - ✓ further elimination of solvent
- *reduced-pressure interface* with skimmer

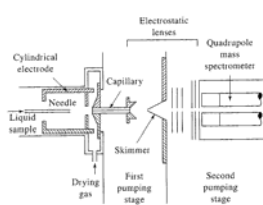
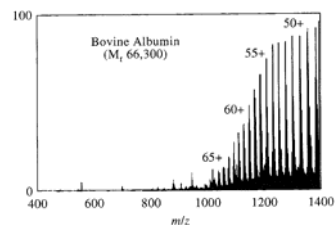


Figure 20-8 Apparatus for electrospray ionization. (from J. B. Fenn et al., Science, 1989, 246, 65)

47

## ESI-MS: Results/Applications

- High Multiple Charges
- Ideal for high-MW biomolecules
  - puts  $m/z$  within common mass range of mass analyzers
- Low Fragmentation



48