# Mass



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Mass spectrometry (Mass Spec or MS) uses high energy electrons to break a molecule into fragments.

 Separation and analysis of the fragments provides information about:
 Molecular weight

Structure

The impact of a stream of high energy electrons causes the molecule to lose an electron forming a radical cation.

A species with a positive charge and one unpaired electron



The impact of the stream of high energy electrons can also break the molecule or the radical cation into fragments.



Background

#### □ Molecular ion (parent ion):

The radical cation corresponding to the mass of the original molecule



The molecular ion is usually the highest mass in the spectrum

- Some exceptions w/specific isotopes
- Some molecular ion peaks are absent.

#### Mass spectrum of ethanol (MW = 46)



SDBSWeb : http://riodb01.ibase.aist.go.jp/sdbs/ (National Institute of Advanced Industrial Science and Technology, 11/1/09)

# □ The cations that are formed are separated by magnetic deflection.



Background

Only cations are detected.
 Radicals are "invisible" in MS.

The amount of deflection observed depends on the mass to charge ratio (m/z).

Most cations formed have a charge of +1 so the amount of deflection observed is usually dependent on the mass of the ion.

The resulting mass spectrum is a graph of the mass of each cation vs. its relative abundance.

 The peaks are assigned an abundance as a percentage of the base peak.
 the most intense peak in the spectrum

□ The base peak is not necessarily the same as the parent ion peak.

#### The mass spectrum of ethanol



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Most elements occur naturally as a mixture of isotopes.

The presence of significant amounts of heavier isotopes leads to small peaks that have masses that are higher than the parent ion peak.

- M+1 = a peak that is one mass unit higher than M<sup>+</sup>
- M+2 = a peak that is two mass units higher than M<sup>+</sup>

# Nitrogen: Odd number of N = odd MW



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#### □ Bromine:





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# Chlorine:

#### M+2 is ~ 1/3 as large as M<sup>+</sup>



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	Relative Intensities			
Halogen	М	M + 2	M + 4	M + 6
Br	100	97.7		
Br <sub>2</sub>	100	195.0	95.4	
Br <sub>3</sub>	100	293.0	286.0	93.4
Cl	100	32.6		
Cl <sub>2</sub>	100	65.3	10.6	
Cl <sub>3</sub>	100	97.8	31.9	3.47
BrCl	100	130.0	31.9	
Br <sub>2</sub> Cl	100	228.0	159.0	31.2
Cl <sub>2</sub> Br	100	163.0	74.4	10.4

#### □ Sulfur:

#### M+2 larger than usual (4% of M<sup>+</sup>)



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- The impact of the stream of high energy electrons often breaks the molecule into fragments, commonly a cation and a radical.
  - Bonds break to give the most stable cation.
  - Stability of the radical is less important.

Alkanes

Fragmentation often splits off simple alkyl groups:

 $\Box$  Loss of methyl $M^+$  - 15 $\Box$  Loss of ethyl $M^+$  - 29 $\Box$  Loss of propyl $M^+$  - 43 $\Box$  Loss of butyl $M^+$  - 57

Branched alkanes tend to fragment forming the most stable carbocations.

Mass spectrum of 2-methylpentane



#### □ Alkenes:

#### Fragmentation typically forms resonance stabilized allylic carbocations





methallyl cation, m/z 55





#### Aromatics:

Fragment at the benzylic carbon, forming a resonance stabilized benzylic carbocation (which rearranges to the tropylium ion)



Aromatics may also have a peak at m/z = 77 for the benzene ring.



#### □ Alcohols

- Fragment easily resulting in very small or missing parent ion peak
- May lose hydroxyl radical or water
  - $\Box M^+ 17 \text{ or } M^+ 18$
- Commonly lose an alkyl group attached to the carbinol carbon forming an oxonium ion.
  - I o alcohol usually has prominent peak at m/z = 31 corresponding to H<sub>2</sub>C=OH<sup>+</sup>

#### □ MS for 1-propanol



Amines

Odd M<sup>+</sup> (assuming an odd number of nitrogens are present)

α-cleavage dominates forming an iminium ion

$$CH_{3}CH_{2}-CH_{2}-N-CH_{2}+CH_{2}CH_{2}CH_{3} \longrightarrow CH_{3}CH_{2}CH_{2}N=CH_{2}$$

$$H$$

$$H$$

m/z = 72

iminium ion



#### Ethers

 $\blacksquare$   $\alpha$ -cleavage forming oxonium ion



Loss of alkyl group forming oxonium ion

$$[R-CH_2-O-R']^{\ddagger} \xrightarrow{\sim H} R-CH=O^{\ddagger}H$$

■ Loss of alkyl group forming a carbocation  $[R-CH_2-O-R']^{\ddagger} \longrightarrow R-CH_2-O + +R'$ 

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not observed

alkyl cation

#### MS of diethylether $(CH_3CH_2OCH_2CH_3)$





#### MS for hydrocinnamaldehyde



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 $\Box$  Loss of R' forming RC $\equiv O$ :



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 $\Box$  Esters (RCO<sub>2</sub>R')

Common fragmentation patterns include:

- Loss of OR'
  - peak at M<sup>+</sup> OR'

□ Loss of R' ■ peak at M<sup>+</sup> - R'



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# Mac-Laferty Rearrangement



Aldehyde



m/e = 92

Alkyl benzene







#### **>**Retro Diels-Alder rearrangement





#### Loss of small molecules, such as H<sub>2</sub>O, CO, C<sub>2</sub>H<sub>4</sub>



#### **>**Four-member ring rearrangement

$$CH_3 - CH_2 - O - CH_2 - CH_3 \xrightarrow{-CH_3} CH_3 - CH_2 - O = CH_2 = H_2C - H_1 - H_2C - H_2 + H_2C - H_2 - H_2C - H_2 - H_2C - H_2$$

$$-C_2H_4$$
 +  
HO=CH<sub>2</sub>

#### >Other rearrangement



$$C_3H_7^+ \longrightarrow C_3H_5^+ + H_2$$

42

#### **Determination of Molecular Mass**

- When a M+ peak is observed it gives the molecular mass assuming that every atom is in its most abundant isotopic form
- Remember that carbon is a mixture of 98.9% <sup>12</sup>C (mass 12), 1.1% <sup>13</sup>C (mass 13) and <0.1% <sup>14</sup>C (mass 14)
- We look at a periodic table and see the atomic weight of carbon as 12.011 - an average molecular weight
- The mass spectrometer, by its very nature would see a peak at mass 12 for atomic carbon and a M + 1 peak at 13 that would be 1.1% as high
- The Nitrogen Rule is another means of confirming the observance of a molecular ion peak

- If a molecule contains an even number of nitrogen atoms (only 'common' organic atom with an odd valence) or no nitrogen atoms the molecular ion will have an even mass value
- If a molecule contains an odd number of nitrogen atoms, the molecular ion will have an odd mass value
- If the molecule contains chlorine or bromine, each with two common isotopes, the determination of M+ can be made much easier, or much more complex as we will see.

#### Rule of Thirteen

The "Rule of Thirteen" can be used to identify possible molecular formulas for an unknown hydrocarbon, C<sub>n</sub>H<sub>m</sub>.

Step 1: n = M<sup>+</sup>/13 (integer only, use remainder in step 2)

Step 2: m = n + remainder from step 1



#### Rule of Thirteen

□ If a heteroatom is present,

- Subtract the mass of each heteroatom from the MW
- Calculate the formula for the corresponding hydrocarbon
- Add the heteroatoms to the formula

# DBE

Double Bond Equivalent =(C+1) -(H-N+X)/2

C = number of Carbon atoms in the compound H = number of Hydrogen atoms in the compound N = number of Nitrogen atoms in the compound X = number of Halogen atoms in the compound

# A compound having formula of

 $C_{9}H_{12}O$ DBE = (9+1)- (12-0+0)/2 = 10-6 = 4 (One ring + 3 double bond)  $C_{8}H_{9}OCl$ DBE = (8+1)-(9-0+1)/2 = 9-5 = 4 (One ring + 3 double bond)

#### Rule of Thirteen

**Example:** A compound with a molecular ion peak at m/z = 102 has a strong peak at 1739 cm<sup>-1</sup> in its IR spectrum. Determine its molecular formula.

- \*9. Calculate the molecular formulas for possible compounds with molecular masses of 136, using the Rule of Thirteen. You may assume that the only other atoms present in each molecule are carbon and hydrogen.
  - (a) A compound with two oxygen atoms
  - (b) A compound with two nitrogen atoms
  - (c) A compound with two nitrogen atoms and one oxygen atom
  - (d) A compound with five carbon atoms and four oxygen atoms

\*6. Determine the index of hydrogen deficiency for each of the following compounds.

- (a)  $C_8H_7NO$  (d)  $C_5H_3CIN_4$
- (b)  $C_3H_7NO_3$  (e)  $C_{21}H_{22}N_2O_2$
- (c)  $C_4H_4BrNO_2$
- \*7. A substance has the molecular formula  $C_4H_9N$ . Is there any likelihood that this material contains a triple bond? Explain your reasoning.
  - 12. A carbohydrate was isolated from a sample of cow's milk. The substance was found to have a molecular mass of 342. The unknown carbohydrate can be hydrolyzed to form two isomeric compounds, each with a molecular mass of 180. Using the Rule of Thirteen, determine a molecular formula and an index of hydrogen deficiency for the unknown and for the hydrolysis products. (*Hint:* Begin by solving the molecular formula for the 180-amu hydrolysis products. These products have one oxygen atom for every carbon atom in the molecular formula. The unknown is **lactose.** Look up its structure in *The Merck Index* and confirm its molecular formula.)



#### **Electron Bomb Ionization (EI)**

Sample is heated and energized by a beam of electrons, usually gives a molecular ion (M+) and a lot of fragments H-C-C+H H H





#### **Chemical Ionization (CI)**

- Electron ionization leads to fragmentation of the molecular ion, which sometimes prevents its detection.
- □ Chemical ionization (CI):
- A technique that produces ions with little excess energy.
- Thus this technique presents the advantage of yielding a spectrum with less fragmentation in which the molecular species is easily recognized.
   Consequently, chemical ionization is complementary to electron ionization.
- $\Box$  Reagent gases like CH<sub>4</sub> and NH<sub>3</sub> so M+CH<sub>4</sub> or M+NH<sub>3</sub> ions peak also observed in spectra



Field ionization (FI) is a method that uses very strong electric fields to produce ions from gasphase molecules.



#### Matrix Assisted Laser Desorption Ionization (MALDI)

- sample is co-crystallized with a matrix and then irradiated with laser.
- MALDI is achieved in two steps. In the first step, the compound to be analyzed is dissolved in a solvent containing in solution small organic molecules, called the matrix. The second step occurs under vacuum conditions inside the source of the mass spectrometer.

# Properties of MALDI

- □ Good solubility
- Vapour pressure must be sufficiently low to maintain vacuum conditions
- Viscosity must allow diffusion of the analyte from the bulk to the surface
- Polar : to solvate and separate preformed ion
- Less Sensitive to Salts
- Lower PRACTICAL detection limits
- Easier to interpret spectra (less multiple charges)
- Quick and easy
- Higher mass detection
- Higher Throughput (>1000 samples per hour)

#### Fast atom bombardment (FAB)

 Softer than EI and CI. Ions are produced by bombardment with heavy atoms. Gives (M+H)<sup>+</sup> ions and little fragmentation.
 Good for more polar compounds

Ar +	e	Ar+	acceleration
(5-15	KeV)		

 $Ar^+ + Ar$   $Ar + Ar^+$ 

fast slow <u>+</u> 8 KeV fast slow

#### **ElectroSpray Ionization (ESI)**

Electrospray is abbreviated to ESI Sample is sprayed out of a narrow nozzle in a high potential field. Generates positive  $(M+nH)_n^+$  and negative  $(M - nH)_n^-$  ions and almost no fragmentation. Generates multiple charged ions.



#### **Properties of ESI**

#### **Advantages**

Electrospray Ionization can be easily interfaced to LC. Absolute signals from Electrospray are more easily reproduced, therefore, better quantitation.

Mass Accuracy is considered better.

Multiple charging is more common then MALDI.

#### Disadvantages

No Fragmentation Need Polar Sample

Need Solubility in Polar Solvent (MeOH, ACN, H2O, Acetone are best) Sensitive to Salts Suppression

#### PROBLEMS

- \*1. A low-resolution mass spectrum of the alkaloid vobtusine showed the molecular weight to be 718. This molecular weight is correct for the molecular formulas  $C_{43}H_{50}N_4O_6$  and  $C_{42}H_{46}N_4O_7$ . A high-resolution mass spectrum provided a molecular weight of 718.3743. Which of the possible molecular formulas is the correct one for vobtusine?
- \*2. A tetramethyltriacetyl derivative of oregonin, a diarylheptanoid xyloside found in red alder, was found by low-resolution mass spectrometry to have a molecular weight of 660. Possible molecular formulas include C<sub>32</sub>H<sub>36</sub>O<sub>15</sub>, C<sub>33</sub>H<sub>40</sub>O<sub>14</sub>, C<sub>34</sub>H<sub>44</sub>O<sub>13</sub>, C<sub>35</sub>H<sub>48</sub>O<sub>12</sub>, C<sub>32</sub>H<sub>52</sub>O<sub>14</sub>, and C<sub>33</sub>H<sub>56</sub>O<sub>13</sub>. High-resolution mass spectrometry indicated that the precise molecular weight was 660.278. What is the correct molecular formula for this derivative of oregonin?
- \*3. An unknown substance shows a molecular ion peak at m/e = 170 with a relative intensity of 100. The M + 1 peak has an intensity of 13.2, and the M + 2 peak has an intensity of 1.00. What is the molecular formula of the unknown?
- \*4. An unknown hydrocarbon has a molecular ion peak at m/e = 84, with a relative intensity of 31.3. The M+1 peak has a relative intensity of 2.06, and the M + 2 peak has a relative intensity of 0.08. What is the molecular formula for this substance?
- \*5. An unknown substance has a molecular ion peak at m/e = 107, with a relative intensity of 100. The relative intensity of the M + 1 peak is 8.00, and the relative intensity of the M + 2 peak is 0.30. What is the molecular formula for this unknown?
- \*6. The mass spectrum of an unknown liquid shows a molecular ion peak at m/e = 78, with a relative intensity of 23.6. The relative intensities of the isotopic peaks are as follows.

<i>m</i> / <i>e</i> = 79	Relative intensity $= 0.79$
80	7.55
81	0.25

Q7. Write the molecular formula and structure of compound by following spectral data

a) The infrared spectrum of this unknown shows a strong peak near 1715  $cm^{-1}$ .



The infrared spectrum of this compound lacks any significant absorption above  $3000 \text{ cm}^{-1}$ . There is a prominent peak near 1740 cm<sup>-1</sup> and another strong peak near 1200 cm<sup>-1</sup>.

(b)



The infrared spectrum of this substance shows a very strong, broad peak in the range of  $2500-3000 \text{ cm}^{-1}$ , as well as a strong, somewhat broadened peak at about  $1710 \text{ cm}^{-1}$ .

C)





