L.7. Mass Spectrum Interpretation

• Fragmentation reactions
• Spectrum interpretation
• Confirmation of ion structural assignment
• Biomolecule dissociation

Fragmentation reactions

1. Fragmentation reactions of positive ions
   a. Odd-electron cations or radical cations (OE⁺)
      ➢ σ dissociation
      ➢ i cleavage
      ➢ α cleavage
      ➢ rearrangement
   b. Even-electron cations (EE⁺)
   c. Parity rule

2. Fragmentation reactions of negative ions
   a. Even-electron anions (EE⁻)
   b. Radical anions (OE⁻)
   c. Charge-remote fragmentation

Refer to: E. De Hoffmann, et. al. Mass Spectrometry Principles and Applications, 1996
1. Fragmentation reactions of positive ions
   a. Odd-electron cations or radical cations (OE⁺)
      ➢ **σ** dissociation: the expulsion of an electron from a **σ** bond

      ![Diagram of σ dissociation]

      One fragment keeps the charge and the other is a radical.

      **Stevenson’s rule**

      The predominant cation is the one corresponding to the radical with the lower ionization potential.

      ![Diagram of Stevenson’s rule]

      ➢ **i** cleavage: the cleavage of the bond adjacent to the heteroatom

      The heteroatom carries the electron away and forms a neutral radical.

      ➢ **α** cleavage

      radical site initiated reaction

      ![Diagram of α cleavage - radical site initiated]

      charge initiated reaction
**i-buty ether**

![Diagram of i-buty ether fragmentation]

Figure 6.5: Fragmentation of i-buty ether.

**ethyl 2-buty ether.**

![Diagram of ethyl 2-buty ether fragmentation]

Figure 6.6: Spectrum of ethyl 2-buty ether.
Competition between i cleavage and α cleavage

I cleavage: large heteroatoms such as sulfur
α Cleavage trend:

Br, Cl < R - π bond, S, O < N

Figure 6.7: Spectra of (top) butylamine, and (bottom) butanethiol.

Figure 6.8: McLafferty rearrangement through a six-atom ring intermediate. Figure 5.4 shows another example and details the rules linking the mass and electron parities.
b. Even-electron cations (EE⁺)

OE⁺⁺ or OE⁻⁻: even mass
EE⁺⁺ or EE⁻⁻: odd mass

an even-electron ion prefers to yield even-electron fragment ions

Parity rule

A stable ion must be formed!
c. Parity rule

The cleavage of the bond that is adjacent to the charged site, while observing a migration of the charge, is common fragmentation process that occurs especially often when it allows the elimination of a small stable molecule.

\[
\begin{align*}
R-OH + H^+ & \rightarrow R-OH_2^+ \\
R-OH_2^+ & \rightarrow R^+ + H_2O
\end{align*}
\]

Common neutral fragments in the order of preference to be lost:
HCl > H₂O > H₂S > CH₃OH > CH₃SH > NH₃

Reactions of even-electron ions that obey the parity rule

1. Cleavage of a bond with charge migration:

2. Cleavage of a bond with cyclization and charge migration:

3. Cleavage of two bonds in a cyclic ion with charge retention:

4. Cleavage of two bonds with rearrangement and charge retention:

H$_2$N(CH$_2$)$_n$OH

Anchimeric assistance:

\[
\begin{array}{|c|c|c|c|c|c|}
\hline
n & 2 & 3 & 4 & 5 & 6 \\
\hline
(M + H) - H_2O (M + H)^+ & 0.42 & 0.21 & 0.20 & 0.15 & 0.07 \\
\hline
\end{array}
\]

n=5

Four-center rearrangement

Iminium ions
short alkyl chain

Iminium ions
long alkyl chain

2. Fragmentation reactions of negative ions

a. Even-electron anions (EE⁻)

H loss

(CH₂COCH₃)⁻ → CH₂COCH₂⁻ + H⁻

alkyl radical loss

Ph⁻CHOCH₃ → PhCHO⁻⁻ + CH₃⁻

anion-neutral complex

CH₂COCOCH₃ → [CH₂CO(CH₂CO)] → CH₃C=O + CH₂CO

Hydride ion complex

CH₃-CH₂-O⁻ → [H⁻ (CH₃-CHO)] → [H₂ (CH₂=CHO⁻)] → (CH₂=CHO)⁻ + H₂

CO₂ loss

CF₃COO⁻ → CF₃⁻ + CO²⁻ Cleavage involving charge migration
Various rearrangements often result from internal nucleophilic condensation, intramolecular nucleophilic substitution, formation of an ion-molecule complex.

Figure 6.12: MS/MS product ions spectrum of the [M – H]⁻ pseudomolecular ion of ethylene glycol diacetate. Almost the same spectrum is observed from ethylene glycol monoacetate. See figure 6.13 for the explanation of the observed masses.

Figure 6.13: Fragmentation scheme of ethylene glycol diacetate and ethylene glycol monoacetate anions. The spectrum is displayed in figure 6.12. These two anions interconvert by the reversible pathway a, as demonstrated by the identity of the spectra of these two compounds. Other steps have been proved to be reversible by labeling experiments. Square brackets indicate intra-molecular complexes.
b. Radical anions (OE⁻)

- single cleavage

\[ R - X - H \rightarrow RX^- \]

\[ Ar - O - R \rightarrow ArO^- \]

\( X = O \) or NR

\[ R - C - O - R' \rightarrow (RCO)_2^- \rightarrow R \rightarrow R - C - OR' \]

\( R = \text{alkyl, aryl} \quad R' = \text{alkyl, aryl or CO alkyl (aryl)} \)

Ortho effect

Loss of HO-

X=O, N

ArS-

[M-C3H8S]⁻
CID mass spectrum of C$_{60}^-$ (m/z 720) using argon as the collision gas at $E_{cm}=190$ eV ($E_{cm}=10$ eV)

- Loss of NO-
- Rearrangement

CID mass spectrum of fullerene, discovered by Kroto $^{19}$, using a mixture of CH$_4$ and N$_2$O as ionizing gas. Small peaks are observed at 734 and 736 Th $^{20}$. Used for MS Short Course at Tsinghua by R. Graham Cooks, Hao Chen, Zheng Ouyang, Andy Tao, Yu Xia and Lingjun Li
c. Charge-remote fragmentation

Fragmentation can occur at a position remote from that where the charge is localized.

Spectrum interpretation

Useful methods:

- Elemental composition
- Molecular mass

In EI: linear saturated hydrocarbons—weak molecular ion
  branched saturated hydrocarbons—no molecular ion
  aromatic hydrocarbons—strong molecular ion
  hydrocarbons containing electronegative saturated heteroatoms (e.g. O, F)—no molecular ion

In softer ionization: saturated compounds—-[M-H]^+
  unsaturated or heteroatom-containing compounds—[M+H]^+

- # of rings and unsaturations
- The relationships between the structure and fragmentations

Determination of chemical formula using exact mass measurement

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<th>Measured</th>
<th>Calculated</th>
<th>Error (× 10^3 d)</th>
<th>Composition</th>
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^The fluorine-containing ions are due to an internal standard.
Isotope peaks also provide useful information about chemical composition.

**Typical ions**

Compounds containing hydrocarbon chains: a series of ions distant from each other by 14 Da (-CH₂⁻)

Molecules with a benzene ring: C₆H₅⁺ (m/z 77), C₆H₇⁺ (m/z 91)

Trimethylsilyl derivatives: (CH₃)₃Si⁺ (m/z 73)
Typical neutral loss

Loss of $H_2$ when it brings about an increased conjugation or aromaticity

![Tetralin spectrum](image)

Figure 6.16: EI spectrum of tetralin. The complete aromatization through the loss of four hydrogen atoms is indicated by the presence of the peak at $m/z$ 128. The most abundant ion results from the loss of ethylene. As in the case of most cyclopanes, an intense ion is observed at ($M - 15$), derived from the loss, following a rearrangement, of a methyl group. The typical ions benzylum (91) and phenylum (77) lose 26 Da, acetylene, and produce fragments at $m/z$ 65 and 51, respectively.

Loss of 15 Da is typical for the elimination of a methyl group $CH_3$

--saturated rings such as cyclohexene

Loss of 16 Da is typical for the elimination of a methane $CH_4$ or an oxygen $O$

--loss of methane from a angular methyl group of steroids or loss of oxygen from N-oxides and sulfoxides

Loss of 18 Da is typical for the elimination of a water molecule $H_2O$, indicating the presence of a hydroxyl group

Loss of 19 Da or 20 Da are typical for the presence of fluorine $F$

Loss of 30 Da – NO loss

Loss of 36 Da – HCl loss

Loss of 46 Da – NO$_2$ loss

Loss of 78 Da – benzene loss
Example I

Figure 6.17: Spectra of three compounds having the same mass illustrating a few interpretative rules.

Example II

Figure 6.18: Comparison of spectra of amines, thiols and a thioether. The amine spectra display only very weak molecular ions. Thiols or thioethers have molecular ions that are relatively abundant, followed by the typical isotopic $^{34}$S (4%) peak.
Loss of alkyl radicals

\[
\text{NH}_2 \rightarrow \text{NH}_2^{+} \quad \text{m/z 58}
\]

\[
\text{NH}_2 \rightarrow \text{H}_2\text{C} = \text{NH}^{+} \quad \text{m/z 30}
\]

\[
\text{NH} = \text{CH}_2 \rightarrow \text{NH}_2 = \text{CH}_2^{+} \quad \text{m/z 30}
\]

Loss of HS radicals

\[
\text{SH} \rightarrow \text{H} + \text{SH}^{+} \quad \text{m/z 57}
\]

\[
\text{SH} \rightarrow \text{CH}_4 + \text{H}^{+} \quad \text{m/z 57}
\]

\[
\text{SH} \rightarrow \text{H}_2 + \text{CH}_4^{+} \quad \text{m/z 41}
\]

\[
\text{SH} \rightarrow \text{m/z 39}
\]

Loss of alkyl radicals

\[
\text{SH} \rightarrow \text{SH}^{+} \quad \text{m/z 75}
\]

\[
\text{SH} \rightarrow \text{H}_2\text{C} = \text{SH}^{+} \quad \text{m/z 47}
\]

\[
\text{SH} \rightarrow \text{m/z 48}
\]

\[
\text{SH} \rightarrow \text{m/z 49}
\]
Confirmation of ion structural assignment

- Dissociation
- Isotope labeling
- Ion/molecule reaction
- Comparison with authentic compounds
- Calculation

Summary of Guiding principles

---Applicable to EI and to even electron ions in CI. Also to MS/MS fragmentation after EI, CI, DI and SI.

- Product ion stability governs pathway of fragmentation, more stable more likely
- Simple cleavages are favored over rearrangements – unfavorable entropy of complex transition states
- Consequences of energy rule
  - Charge goes to most electropositive element N > O
  - Ordinary octet rule applies ammonium > carbenium
  - Electron unpairing unlikely
    - Odd electron -> odd
    - Odd electron -> even
    - Even electron -> even
  - BUT Even electron -> odd is not
  - Ion as the high enthalpy species dominates over radical in determining thermochemistry
  - Ter > Sec > Prim due to charge “solvation”
  - Similarly longer chain > shorter chain in charge stabilization
  - But radical energy does matter e.g. CH₂ loss almost never occurs
- Consequences of entropy consideration
  - Only if rearrangements give very stable products can they compete against simple cleavages. But they can often do so
    - hence at very low energy we have Rearr.; high energy, we have Simple Cleavage.
  - Hence crossing of rate constant vs. energy curves for Rearr. and Simple Cleavage important feature of Mass spectrometry.
- Further fragmentation follows the same rules. Energy is redistributed and finally low mass products are formed.
- Information is concentrated at higher mass in the primary fragmentations (“neutral losses”). However, there are characteristic ion series at lower mass that also give insights into molecular structure.
- Main fragmentations by functional group
  - Ether type, C-C-X: α-cleavage (α is 1st C-C bond after functionality) -> C=X⁺ + R
  - Ketone type, R₁-C(α)=X: α-cleavage (α is 1st C-C bond after functionality) -> (R₁)C=X⁺ + R₂
  - Further fragmentation: loss of alkene(s) until HC=O⁺ (m/z 29) ketones and H₂C=O·H (m/z 31) ethers