Mass Spectrometry: A Perspective

(Student reference)

Mass Spectrometry

Definition

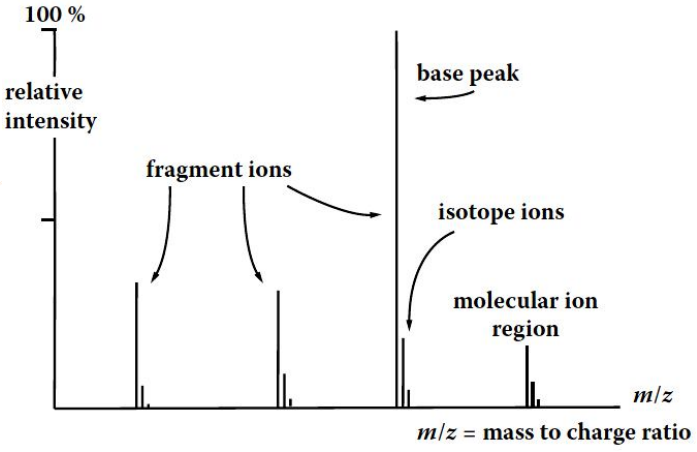
Mass Spectrometry is a powerful analytic technique to assess the relative masses of molecular ions and fragments by utilizing the degree of deflection of charged particles (ions) in vacuum as a result of exerting forces using magnetic and electric fields.

Only charged or ionized compounds are being analyzed in mass spectrometer. The neutral particle or radical remains undetected directly by mass spectrometer. The mass of a molecule is expressed by m/z value. The mass spectrum is obtained by plotting relative abundance in Y-axis against mass/charge ratio in X-axis.

Purpose

a) Determination of molecular mass
b) Structural information of an unknown compound
c) Molecular formula from exact molar masses
d) Isotopic abundance
e) Purity of the sample

Mass spectrum: A mass spectrum is represented by following way:



INSTRUMENTAL METHODS OF CHEMICAL ANALYSIS
Mass Spectrometry

Q. Why is mass 'spectrometry' used instead of 'spectroscopy'?

Unlike other spectroscopy (UV, IR, NMR), it neither involves the absorption of electromagnetic radiation nor does it measure the interactions between the molecules with the spectrum of energy found in electromagnetic spectrum. (However, the output of the instrument has all other spectroscopic characteristics showing an array of signals corresponding to a spectrum of energies).

Mode of ionization

There are several modes of ionization of molecules in mass spectrometry:

1. Gas-Phase Ionization
   Electron Ionization (EI)
   Chemical Ionization (CI)
   Desorption Chemical Ionization (DCI)
   Negative-ion chemical ionization (NICI)
2. Field Desorption and Ionization
   Field Desorption (FD)
   Field Ionization (FI)
3. Particle Bombardment
   Fast Atom Bombardment (FAB)
   Secondary Ion Mass Spectrometry (SIMS)
4. Atmospheric Pressure Ionization
   Electrospray Ionization (ESI)
   Atmospheric Pressure Chemical Ionization (APCI)
5. Laser Desorption
   Matrix-Assisted Laser Desorption Ionization (MALDI)

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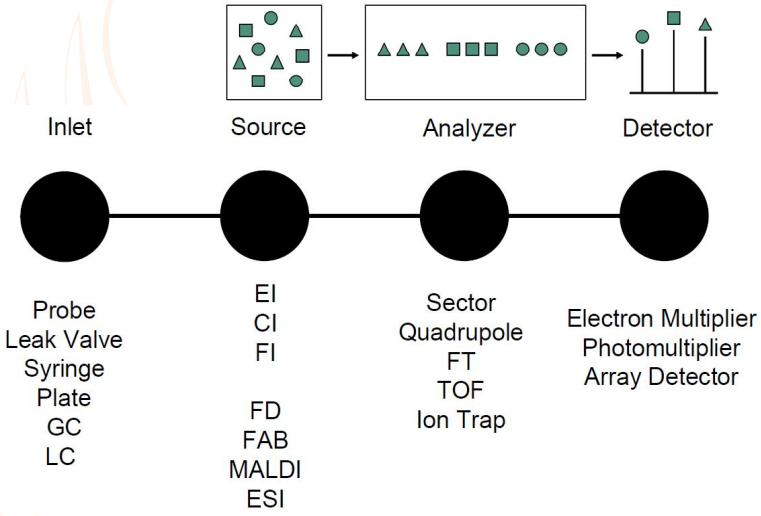
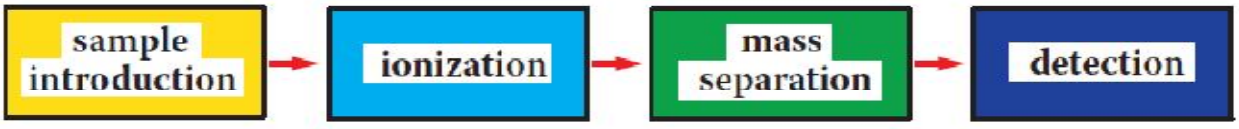
Q. Why is needed different modes of ionization?

A mass spectrometer works by combination of electric and magnetic fields to exert forces on charged particles (ions) in a vacuum. A sample compound should be charged or ionized to be analyzed by a mass spectrometer. The ions must be introduced in the gas phase into the vacuum system of the mass spectrometer. This is easily done for gaseous or heat-volatile samples. However, many (thermally labile) analytes decompose upon heating. These kinds of samples require either desorption or desolvation methods in order to be analyzed by mass spectrometry. The ionization and desorption/desolvation are usually separate processes. The term "ionization method" is commonly used to refer to both ionization and desorption (or desolvation) methods.

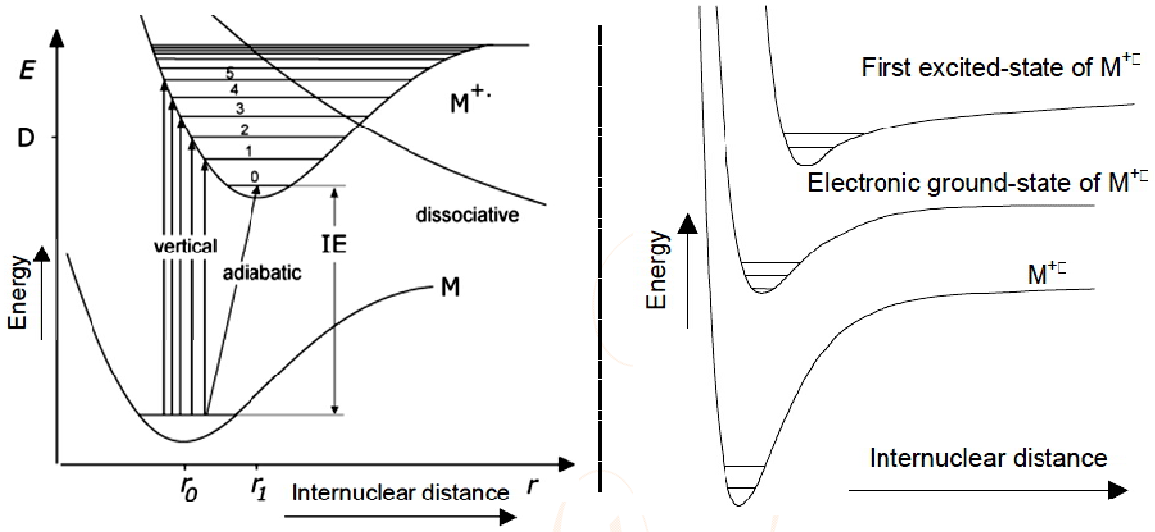
Common parts of mass spectrometer



Basic sequence of events in a mass spectrometer



An electron about 50 eV of kinetic energy with a speed of 4.2×10^8 cm/sec can passes a molecule in about 10^{-16} seconds. The passing electric field induces the valence electrons of the orbit to such an extent that it allows one valence electron to loose and ionization occur. Interestingly, only one negative ion is produced with respect to 10^3 to 10^4 of the positive ions.



The ionization follows Franck-Condon transition in which the positions of the nuclei remain unaltered during ionization. It is thus a vertical ionization that requires higher ionization energy than the adiabatic process. As a result a molecular-ion is formed with several possible vibrational states. Even, some fragmentations occur because of exceeding the dissociation energy. It is also possible to get higher electronic state of molecular-ions that brings about the dissociation even easier, owing to decrease of the depth in the potential energy-curve and drifting of minima to higher internuclear distance. Thus, the fragmentation processes of the molecular ion are induced from the same electronic states in which vibrational and rotational energy are accumulated. The whole process of ionization typically takes 10^{-10} sec within which the energy is redistributed to a few vibrational levels for

ensuing fragmentation. The ion then returns to the ground electronic state after 10^{-8} s through radiation and there will be no longer fragmentation. This occurs well before the ion leaves the source.

Events	Time scale
Electron ionization	**low femtosecond time scale (10^{-14}-10^{-16} sec)**
Bond vibration	**low picosecond time scale (10^{-10}-10^{-14} sec)**
Bond cleavage	**pico- to nanoseconds (10^{-9}-10^{-12} sec)**
Rearrangements	**nano- to low microsecond time scale (10^{-6}-10^{-9} sec)**
Residence time in ion source	**about one microsecond (10^{-4}-10^{-6} sec)**
Residence time in mass analyzer	**tens of microseconds (10^{-4}-10^{-5} sec)**

The reactions consist of unimolecular fragmentations and depend on the structure and the energy contained within the molecule. Recombinations are ruled out since collisions are non-existent under the usual conditions. However, ring opening and rearrangements are feasible from a fragmentation.

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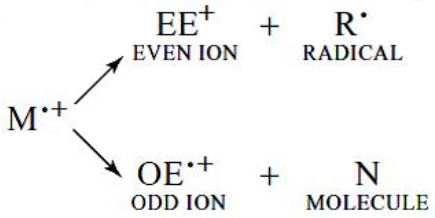
Basic Principles: Electron ionization (EI) and sector analysis

In a mass spectrometer, an organic molecule is initially bombarded with stream of electrons emitted from filament upon heating to several thousand degrees Celsius. The organic molecule is converted into highly energetic positively charged ions. This radical cation which is produced after losing one electron from the molecule is called molecular ion or parent ion ($M^{+\cdot}$). After that, the parent ion undergoes smaller fragmentation; called fragment ions or daughter ions. This whole electron ionization process in gas phase is represented as follows:

Step 1: $M + e^- \longrightarrow M^{+\cdot} + 2\ e^-$ (molecular ion/parent ion)

Step 2: $M^{+\cdot} \longrightarrow m_1^+ + m_2^{\cdot}$ **Or** $m_1^{+\cdot} + m_2$ (fragment ion/daughter ion)

or it can be represented as

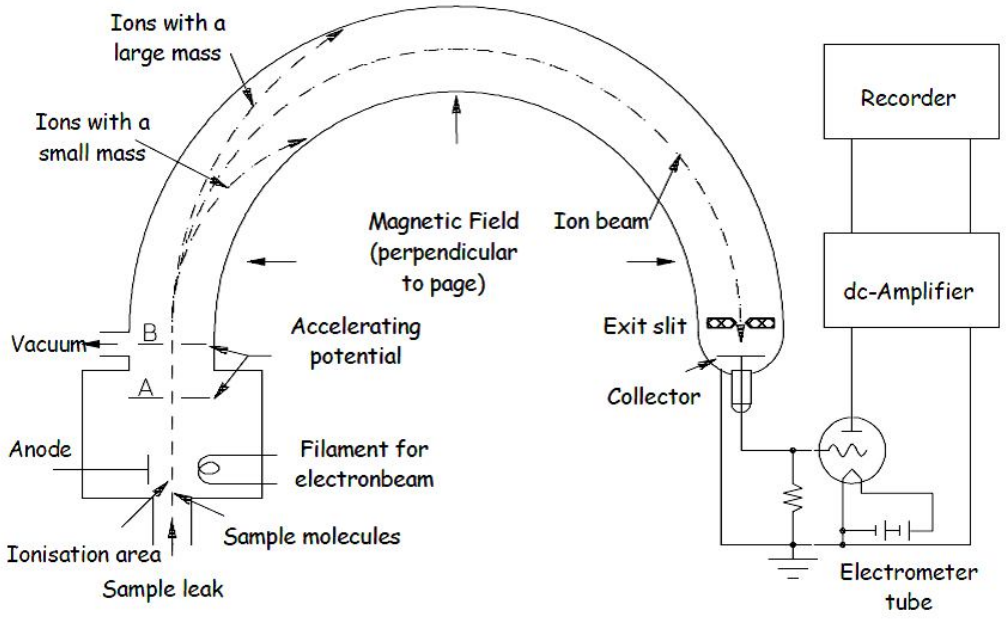


$M + e^- \longrightarrow M^{-\cdot}$ may be possible in step I. $[M^{+\cdot} + 2\ e^-]$ formation is more probable by a factor of 10^2; and positive ion mass spectrometry is the result.

A mass spectrum is formed after all these ions are separated in the mass spectrometer according to their mass-to-charge ratio. Ion abundance versus mass-to-charge ratio is plotted in mass spectrum. The most intense peak is called the base peak and is arbitrarily assigned the relative abundance of 100 %. The abundances of all the other peaks are given their proportionate values, as percentages of the base peak. The relative abundance is commonly used to refer to the number of ions in the mass spectra. When m is given as

the relative mass and z as the charge number, both of which are unitless, m/z is used to denote a dimensionless quantity.

Schematic representation of Mass Spectrometer



The three essential functions of a mass spectrometer:

1. Ion Source: A small sample is ionized, usually to cations by loss of an electron.

2. Mass Analyzer: The ions are sorted and separated according to their mass and charge.

3. Detector: The separated ions are then measured, and the results displayed on a chart.

According to their mass and charge, the molecular ion ($M^{+\cdot}$), fragment ions (m_1^{+}) and fragment radicals ions ($m_1^{+\cdot}$) are separated by deflection in a variable magnetic field. It generates a current (ion current) at the collector in proportion to their relative abundance. The mass spectrum is obtained by plotting relative abundance (Y-axis) against mass/charge ratio (X-axis).

Either a singly charged (m_1^+) or doubly charged ion ($2m_1^{++}$), they appear in the mass spectrum in the same value [2m/2z = m/z]. However, due to lower mass (m_1^+), the singly charged ions are more easily deflected in presence of magnetic field. On the other hand, the neutral particle (m_2) or radical ($m_2^{\cdot}$) remains undetected directly by mass spectrometer. Most of the molecular ions are in highly excited states and undergo rapid fragmentation within the ion source. Therefore, EI mass spectra exhibit a high degree of ion fragmentation. The electrons do not 'impact' molecules. For this reason, it is recommended that the term electron impact must be avoided.

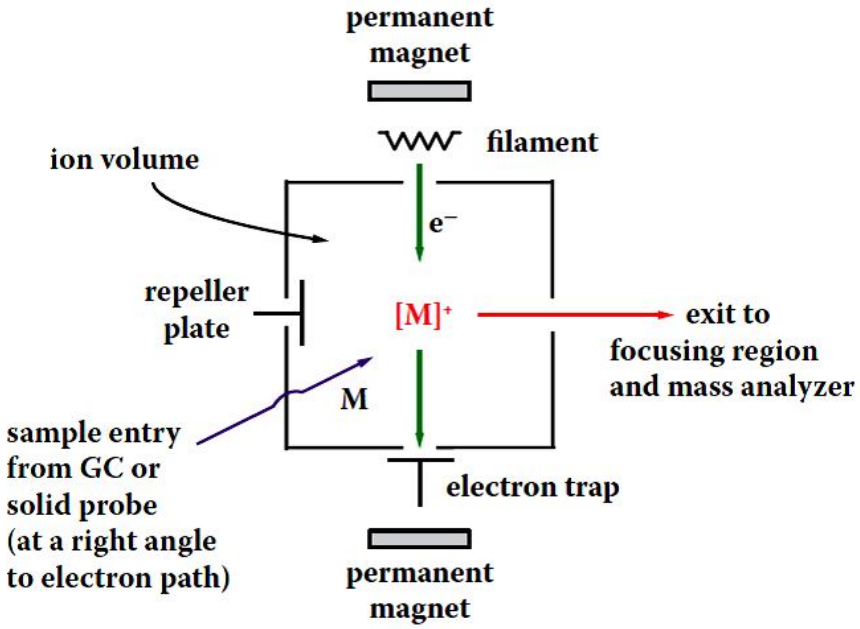
Stable - metastable - unstable ions

a) Stable ions reach the detector without any fragmentation ($k < 10^5\ s^{-1}$)

b) Metastable ions decompose in transit ($10^5\ s^{-1} < k < 10^6\ s^{-1}$)

c) Unstable ions dissociate still within the ion source ($k > 10^6\ s^{-1}$).

A simplified diagram for EI mass technique is illustrated below.



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Measurement principles

1) Very little amount of organic compound (typically one micromole or less), is evaporated and the vapor is dripping into the ionization chamber under the pressure of about 10^{-7} mbar.
2) A heated cathode, the filament typically made of rhenium or tungsten reaches up to 2000 °C and produces the ionizing electrons beam by thermionic emission. The vapor molecules are now ionized by an electron beam. Ionization is achieved by inductive effects rather than strict collision leading to the formation of positive charge by loss of valence electrons.
3) By applying small positive charge (several Volts) from repeller or opposing exit-slit (A), the positive ions are forced out of the ionization chamber. After leaving the ionization chamber, ions are accelerated by an electrostatic field (A>B) of several hundreds to thousands of volts before they enter the analyzer.
4) The separation of ions (large vs. small m/z value) takes place in the analyzer at a pressure of about 10^{-8} mbar. This is achieved by applying a strong magnetic field 1^{r} to the motional direction of the ions. The fast moving ions will follow a circular trajectory (due to the Lorenz acceleration) whose radius is determined by the mass/charge ratio of the ion and the strength of the magnetic field. Ions with different mass/charge ratios are forced through the exit-slit by variation of the accelerating voltage (A>B) or by changing the magnetic-field force.

5) After the ions have passed the exit-slit, they collide on a collector-electrode. The resulting current is amplified and registered as a function of the magnetic-field force or the accelerating voltage.

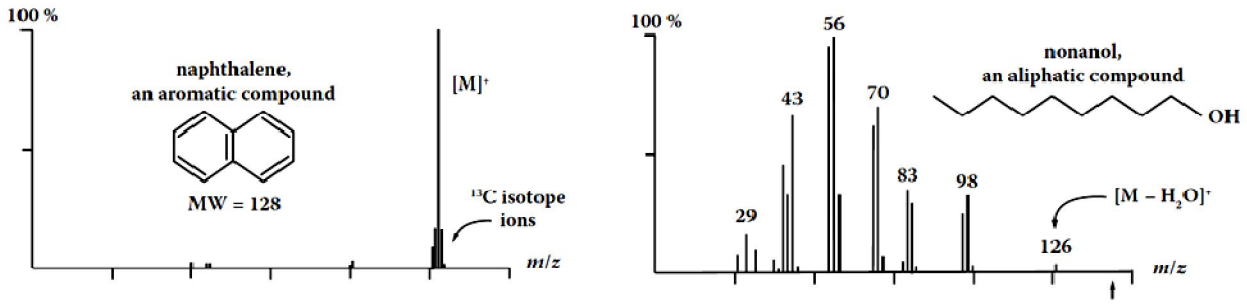
Benefits of EI

- well-understood
- can be applied to virtually all volatile compounds
- reproducible mass spectra
- fragmentation provides structural information
- libraries of mass spectra can be searched for EI mass spectral "fingerprint"

Limitations of EI

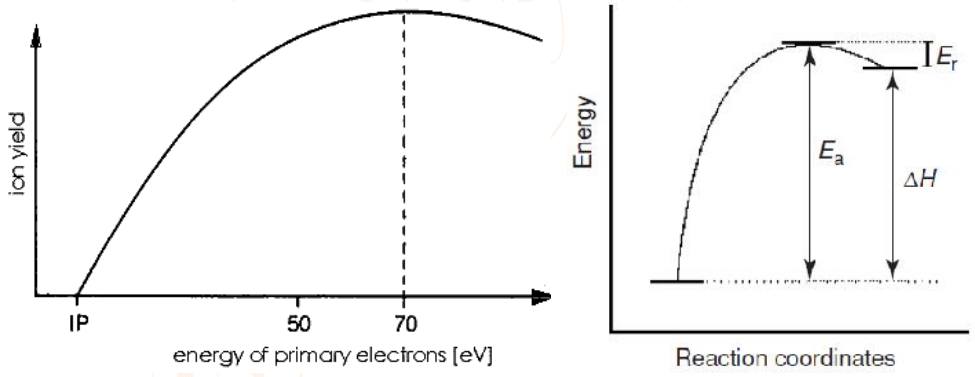
- sample must be thermally volatile and stable
- the molecular ion may be weak or absent for many compounds.
- Typical mass range below less than 1,000 Da.

A comparison between aromatic and aliphatic compounds helps us to understand EI fragmentation. A polynuclear aromatic hydrocarbons e.g naphthalene, exhibits only the molecular ion (left) despite it absorbs considerable excess of energy that may lead to the breaking of bonds to form variety of fragments and rearranged ions. In contrary, saturated aliphatic compound, e.g. nonanol, results extensive fragmentation with no molecular ion peak rather fragment ions are observed.

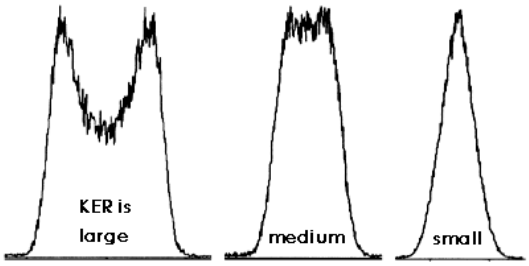


Q. How is ionization potential related in EI MS?

Ans: The energy needed to remove one or more electrons from outermost shell of a neutral atom (or molecule) to form a positively charged ion (cations) in gas phase is called **ionization potential (I.P)**. Most of the organic molecules have their I.P in the range of ***8 to 15 electron volts (eV) (≈ 770 kJ mol^{-1}or 180 KCal mol^{-1} to 1450 kJ mol^{-1}or 350 KCal mol^{-1})***. In EI mass spectrometry, the energy carried by an electron to bombard on a neutral organic molecule to generate cation is ***50-70 eV (standard 70 eV is used equivalent to ≈ 6.75 x 10^{3} kJ mol^{-1} or 1610 KCal mol^{-1})*** which is greater than I.P of common organic molecules. Thus, the excess kinetic energy (70-15 =55 eV ≈ 1270 ***Kcal mol^{-1}*** or 70-8 = 62 EV ≈ ***1430 Kcal mol^{-1}***) imparted to the sample molecules leads to significant fragmentation of molecular ions.



Although most ion fragmentations are endothermic, a significant amount of energy is redistributed as vibrational energy and some energy is converted into translational motion which is released for cleavage of bond to give fragments. This is termed as **kinetic energy release (KER)**. According to the principle of micro-reversibility, the fragmentation activation energy is equal to the endothermicity $\Delta H = E_a - E_r \approx E_a$.



In the extreme right, the narrow Gaussian-shaped peak can be expected to result from a homolytic bond cleavage or an ion-molecule complex-intermediated process because such reactions exhibit small KERs.

[Note: The primary electron energy of 70 eV is set as standard in EI mode because the ionization efficiency is nearly optimum at 70 eV for most of the organic molecules and easy comparison of spectrum with that from other instruments].

Q. Which is the likeliest orbital in organic molecule vulnerable to lose electron upon bombardment around I.P 10-15 eV or 10^3 kJ mol^{-1}?

Ans: If the electron bombardment is carried out around I.P 10-15 KeV or 10^3 kJ mol^{-}, the following likeliest orbital would undergo electron lose.

- The highest occupied orbitals of aromatic systems
- nonbonding orbitals on oxygen and nitrogen atoms
- *the* π-electrons double and triple bonds

It follows Frank Condon principle, where these ionizations occur before structural changes. For that, the ionization of a C-C sigma (σ) bond is much easier than that of C-H bonds.

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The electrons in a molecule are not usually localized. A general view can be conferred that an electron being expelled from the whole molecular orbital and the excitation energy being spread throughout the molecule. Any specificity in the site of electron removal from organic molecule may be lost entirely when electron bombardment of complex being taken place about 70 eV (6×10^3 kJ mol^{-1}).

Q. Why is molecular ion peak usually not observed in EI MS?

Ans: Under EI-MS mode, some compounds fragments so fast that the life time of the molecular ion is too short to be detected by analyzer. The determination of molecular mass is difficult. It is also difficult to analyze high molecular weight of the compounds such as peptides, proteins and other biomolecules.

Q. What is the difference between nominal mass, isotopic mass, and relative atomic mass and calculate the mass of CH_3Cl that might appear in mass spectrum?

- The nominal mass is defined as the integer mass of the most abundant naturally occurring stable isotope of an element. The nominal mass of an element is often equal to the integer mass of the lowest mass isotope of that element, e.g., for H, C, N, O, S, Si, P, F, Cl, Br, I.
- The isotopic mass is the exact mass of an isotope. It is very close to but not equal to the nominal mass of the isotope.
- The relative atomic mass is calculated as the weighted average of the naturally occurring isotopes of an element.

Let us calculate the mass of CH_3Cl that might appear in mass spectrum.

- Chlorine atom has two isotopes of exact masses 34.968852u and 36.965903u with relative abundances 75.77 % and 24.23 % respectively.
- The atomic weight of chlorine atoms is the balanced average: (34.968852 × 0.7577 + 36.965903 × 0.2423) = 35.453 Da.
- The average mass of CH_3Cl is 12.011 + (3 × 1.00794) + 35.453 = 50.4878 Da.
- The monoisotopic mass of CH_3Cl is 12.000000 + (3 × 1.007825) + 34.968852 = 49.992327 u.
- In mass spectrometer, two mass-to-charge ratios will be observed for CH_3Cl due to two isotopic peaks. The first peak will be at m/z (12.000000 + 3 × 1.007825 + 34.968852) = 49.992327 Th, (rounded to m/z 50) and the second peak will be at (12.000000 + 3 × 1.007825 + 36.96590) = 51.989365 Th, (rounded to m/z 52). [Note: 1 Th = 1 u/e; 1 u = 1 Da].

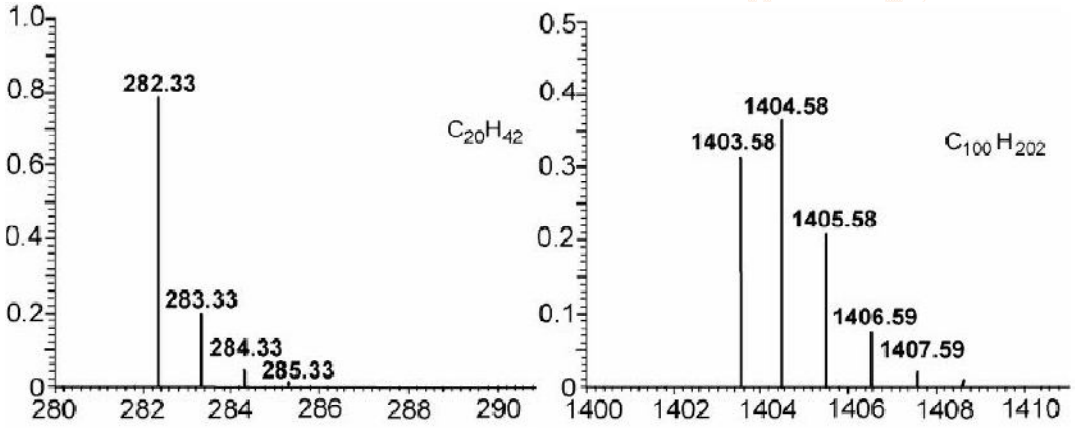
Q. Both HCHO and C_2H_6 have similar integral mass value 30. How is differentiated by MS?

The accurate mass of HCHO and C_2H_6 are 30.010565 and 30.046950 respectively which can be distinguished by high resolution mass spectrometry. Besides, an alternative way of determining mass that utilizes low resolution spectra based on the measurement of intensities of isotopes peaks. It is possible provided the intensity of molecular ion peak is high; otherwise the isotopic peak will be too

Formulae	M + 1	M + 2
$C_2H_4N_2O_4$	3.15	0.84
$C_2H_6N_3O_3$	3.52	0.65
$C_3H_{12}N_4O$	5.00	0.31
$C_4H_{12}N_2O_2$	5.36	0.52
$C_6H_6N_3$	7.72	0.26
C_3H_{12}	9.92	0.44

weak to characterized. For example, the isotope peak ratio of CH_4 is $^{12}CH_4$: $^{13}CH_4$ = 100: 1.08; meaning the intensity of (M+1) peak is 1.08% of the molecular ion peak.

Conclusion: The use of nominal mass is not recommended. The monoisotopic mass is used to distinguish the isotopes experimentally, whereas the average mass is used when the isotopes are not distinguishable. The following alkanes show all types of masses.



Q. What is the role of repeller plate?

Ans: A repeller plate, which carries low positive electrical potential, directs the newly created ions towards a series of accelerating plates and subsequently prevents those cations to move backward.

Q. What is mass defect?

The difference between the exact mass and the integer mass is termed as mass defect. The exact mass of an isotope or a complete molecule is lower than the corresponding nominal mass. The term mass deficiency is also used to describe this deviation. For example, ^{16}O, represents the isotopic mass = 15.994915 u, which is 0.005085 u deficient to the nominal value.

Q. By lowering primary electron energy, does it concern to electron ionization (EI) mass spectra?

Fragmentation pattern carries quite a few structural information of the compound. However, lowering primary electron energy can reduce the number of fragmentations and higher relative intensity of the molecular ion peak in electron ionization (EI) mass spectra. Consequently, simplified spectra would be evident.

Q. State the nitrogen rule in context of mass fragmentation with examples.

Nitrogen rule: A molecule possessing an even number of nitrogen atoms (0, 2, 4, …) will exhibit its monoisotopic molecular ion at an even-numbered m/z whereas with an odd number of nitrogens (1, 3, 5, …) will be detected by an odd-numbered m/z.

Rule: Cleaving off a radical (that contains no nitrogen) from any ion changes the integer m/z value from odd to even or vice versa. Loss of a molecule (that contains no nitrogen) from an ion produces even mass fragments from even mass ions and odd mass fragments from odd mass ions. The rule is rationalized by taking an example of methane ionization.

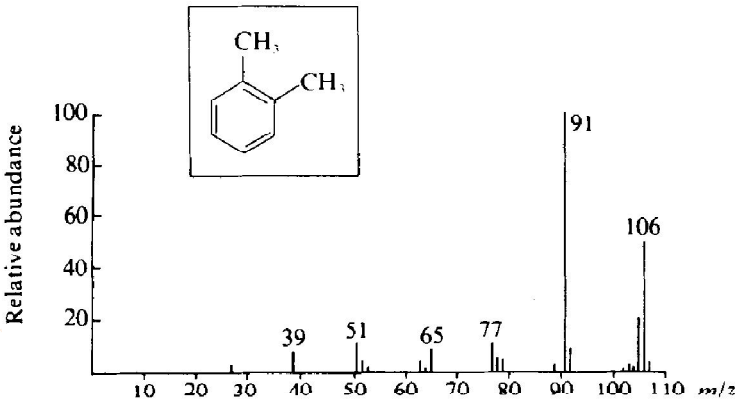
$$CH_4^{+\bullet} \rightarrow CH_3^+ + H^\bullet;\ m/z\ 16 \rightarrow m/z\ 15$$
$$CH_4^{+\bullet} \rightarrow CH_2^{+\bullet} + H_2;\ m/z\ 16 \rightarrow m/z\ 14$$
$$CH_2^{+\bullet} \rightarrow C^{+\bullet} + H_2;\ m/z\ 14 \rightarrow m/z\ 12$$
$$CH_2^{+\bullet} \rightarrow CH^+ + H^\bullet;\ m/z\ 14 \rightarrow m/z\ 13$$
$$CH_3^+ \rightarrow CH^+ + H_2;\ m/z\ 15 \rightarrow m/z\ 13$$

Even number m/z	Odd number m/z
C_3H_8, $C_6H_{12}O_2$, $C_8H_{20}N_2$, $C_9H_{11}ClN_4O_2$ $C_{17}H_{32}Cl_2$,	$C_6H_{11}NO$, $C_{32}H_{25}N_3O_2S$, C_7H_9N, C_3H_6BrN

Location of charge and primary dissociation in molecular ions.

The idea of charge location and the structure and its stereochemistry of the molecule play an essential role in dissociation. After ionization the charge will be located on the element with the smallest ionization potential. Since the molecular ion is a radical cation (un-paired electron system), its dissociation will also form most stable cation (with a paired electron system).

Recognition of molecular ions: In mass spectrometry, along with molecular ion peak, say 'M', the peak at m/z of (M+1), (M+2) , (M+3) etc are also observed. The latter are isotopic peak which arises due to normally presence of many elements in organic molecules which are not monoisotopic. Similarly, peak at m/z of (M-1), (M-15), (M-18) also arise from loss of H, CH_3, H_2O respectively. The peaks are usually sharp and appear as integral value in the mass spectrum. Majority of the ions are singly charged and doubly charged are uncommon. Metastable peak is broad and low intense which gives valuable information about the mode of fragmentation.



Intensity of the molecular ion. The lower the energy required for ionisation of the molecule, and the more stable the molecular ion, the more intense will be the peak in the mass spectrum. Structural features within the molecule have characteristic values of ionisation energy and hence determine the amount of energy required to form the molecular ion. gives a general indication of the intensity of the molecular ion for various types of compounds. It must be borne in mind that if the molecule contains a readily cleaved bond the molecular ion peak will be much less intense. In general the intensity of the molecular ion increases with unsaturation and with the number of rings, but decreases with chain branching. The presence of heteroatoms with easily ionised outer-shell electrons increases the intensity of the molecular ion.

Strong	Medium	Weak or non-existent
Aromatic hydrocarbons	Aromatic bromides and iodides	Aliphatic alcohols, amines and nitriles
Aromatic fluorides, chlorides, nitriles and amines	Conjugated alkenes	Branched chain compounds
Saturated cyclic compounds	Benzyl and benzoyl compounds	Nitro compounds
	Straight chain ketones and aldehydes, acids, esters, amides	
	Ethers	
	Alkyl halides	

Fragmentation: The ion produced by the decomposition of molecule is the fragmentation. The pattern of fragmentation gives an idea about the location of bond breaking and the intensity of that ion. Odd electrons are more relevance than even electron ions of similar mass or abundance since they are generally formed via rearrangement reaction of particular class of compounds. Moreover, ions of high mass are likely to give more information than low mass since they are formed as a result of simple rotational fragmentation. Metastable ions also render the nature of fragmentation process.

Intensity of fragment ions = f (stability of ion and energy relationship of the bond breaking).

The fragmentation eventually leads to carbocation formation which stability in mass spectrum is to be concerned.

Stability: tertiary > secondary > primary > methyl

Even-Electron Rule

Odd-electron ions (such as molecular ions and fragment ions formed by rearrangements) may eliminate either a radical or an even electron neutral species, but even electron ions (such as protonated molecules or fragments formed by a single bond cleavage) will not usually lose a radical to form an odd-electron cation. In other words, the successive loss of radicals is forbidden.

$$[\text{odd}]^{+\cdot} \longrightarrow [\text{even}]^{+} + R\cdot$$

$$[\text{odd}]^{+\cdot} \longrightarrow [\text{odd}]^{+\cdot} + n$$

$$[\text{even}]^{+} \longrightarrow [\text{even}]^{+} + n$$

$$[\text{even}]^{+} \not\longrightarrow [\text{odd}]^{+\cdot} + R\cdot$$

Odd electrons: $CH_4^{+\bullet} \rightarrow CH_3^{+} + H^{\bullet}$ **Even electrons**: $CH_3^{+} \rightarrow CH^{+} + H_2$

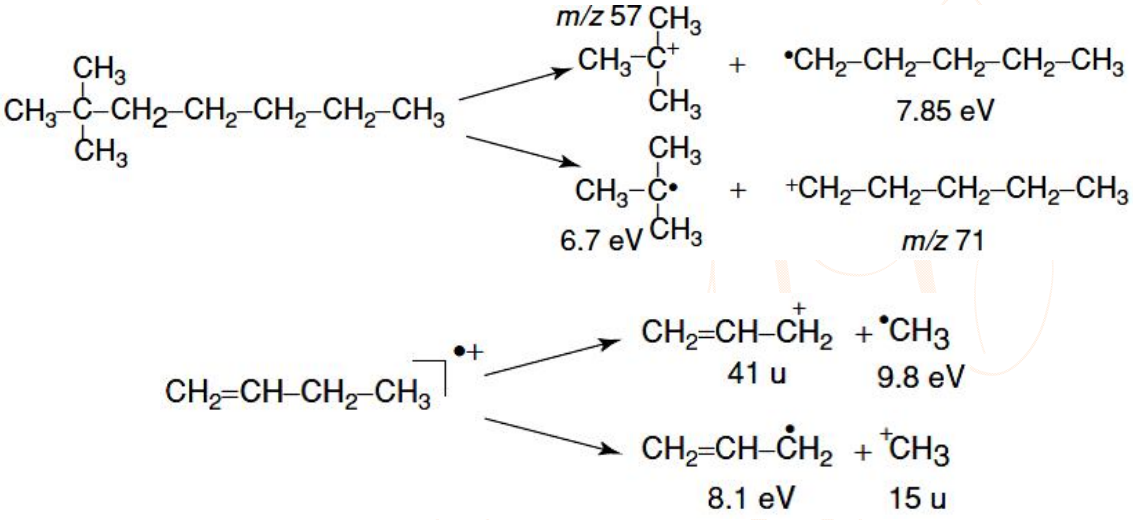
Ex #1: Direct dissociation (σ-cleavage)

The ionization leading to expulsion of an electron from an σ—bond is called direct dissociation. It produces stable carbocation with ejection of the largest possible group as radical.

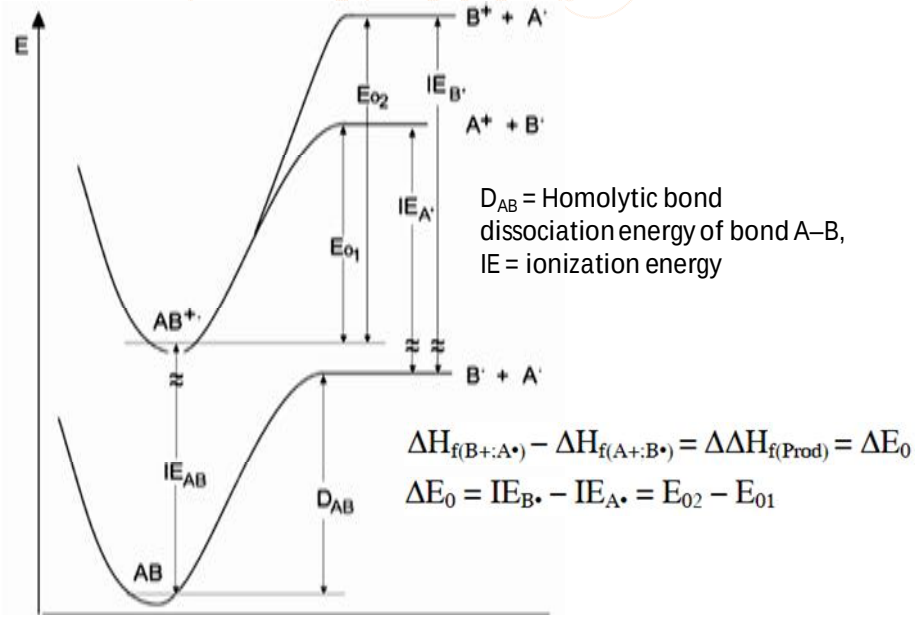
$$R{-}R' + e^- \longrightarrow R{-}R'^{\bullet+} + 2e^-$$

$$R{-}R'^{\bullet+} \begin{cases} \nearrow R^{\bullet} + R'^{+} \\ \searrow R^{+} + R'^{\bullet} \end{cases}$$

According to **Stevenson's rule**, if two charged fragments are in competition to produce a neutral radical by electron attachment, the radical having the highest ionization energy will be produced. This means the positive charge remains on the fragment with the lowest ionization energy.



Thermochemical description of Stevenson's rule



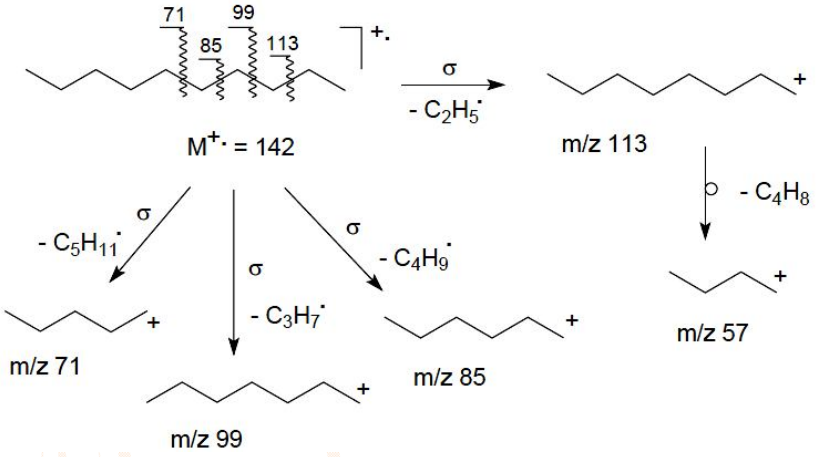
The radical with lower ionization energy will dominate among the products. For example, in case of the α-cleavage of acetone, $CH_3C^{\bullet}=O$ has a by 2.8 eV

lower IE than $^{\bullet}CH_3$. Dissociation leads to energetically more demanding pair of fragments.

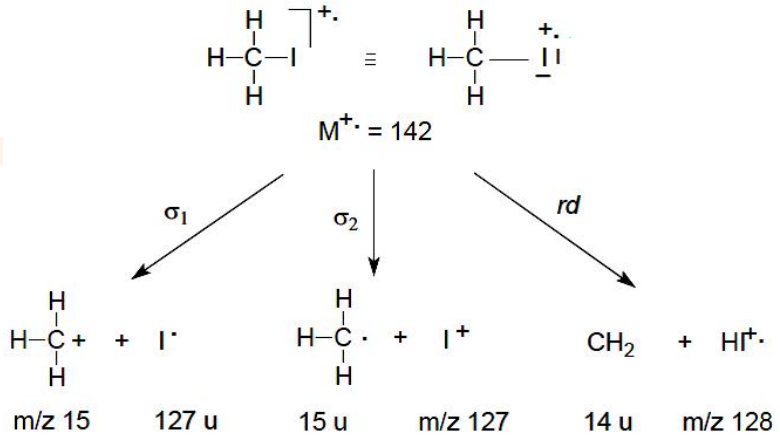
Radical	IE[b] [eV]	Radical	IE[b] [eV]
$H^{\bullet}$	13.6	$CH_3O^{\bullet}$	10.7
$^{\bullet}CH_3$	9.8	$^{\bullet}CH_2OH$	7.6
$^{\bullet}C_2H_5$	8.4	$CH_3C^{\bullet}=O$	7.0

 # Cleavage of Non-Activated Bonds

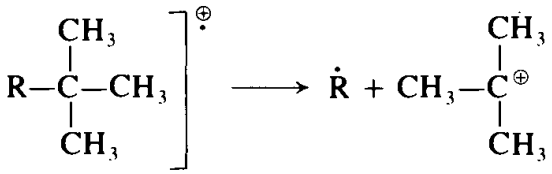
The primary step of dissociation encompasses unspecific σ—bond cleavage at any C_1-C_2 bonds with negligible methyl loss due to the unfavorable thermodynamics of this process.



EI fragmentation of iodomethane is shown below. The relative electronegativities of the halogens their intensities follow the order $I^+ > Br^+ > Cl^+ > F^+$.

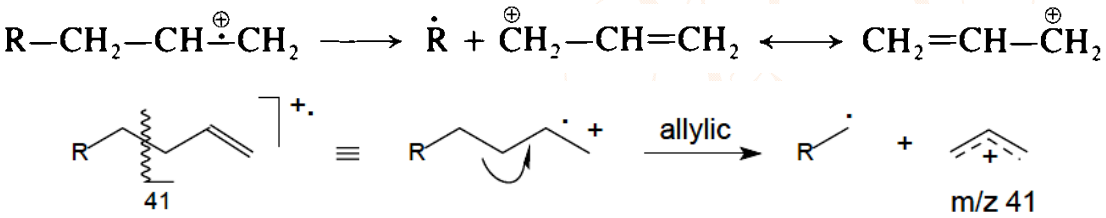


Ex #2: Formation of a stable tertiary carbocation



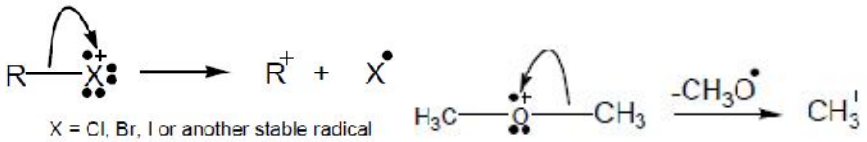
Ex #3: Formation of allylic carbocation

The cleavage of the allylic bond in the alkene molecular ions can be treated analogously to α—cleavage. The double bond is the weakest cleavage-directing functional group.

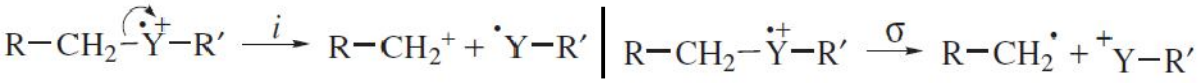


Ex #4: Heterolytic cleavage

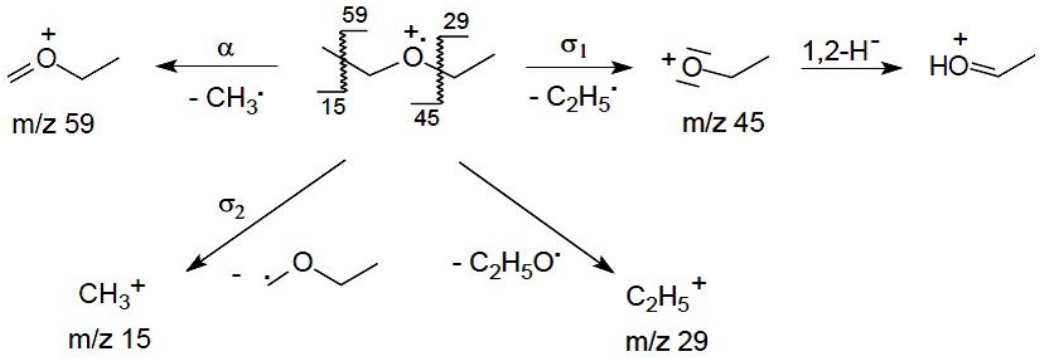
The bond adjacent to a heteroatom is cleaved by a charge-site-initiated reaction or by an induced cleavage (i) as a result of difference in electronegativity. Molecular ions are generally of low intensity.



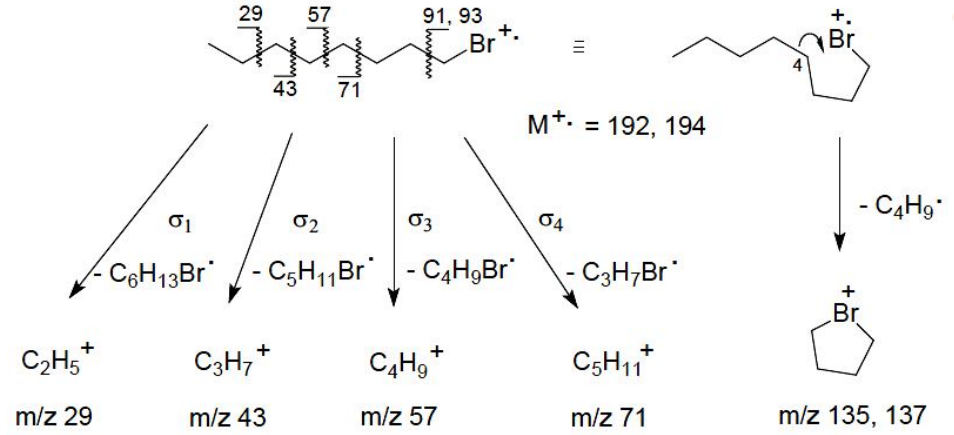
In principle, Stevenson's rule still applies. The cleavage of the adjacent bond could involve radical migration and charge retention if the ionization energy of •YR′ is less than that of $RCH_2^{\bullet}$.



#The fragmentation of diethyl ether.



#The fragmentation of 1-bromo octane.

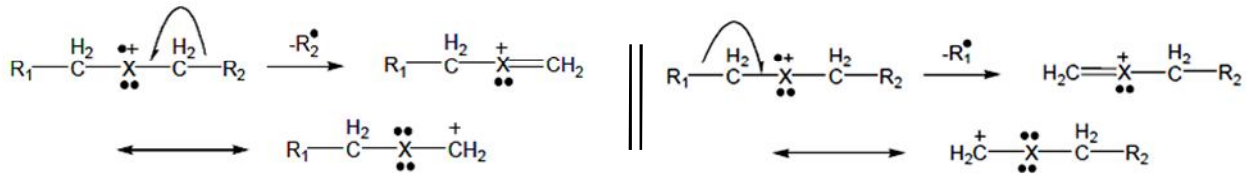


Ex #5: Cleavage of the α—bond

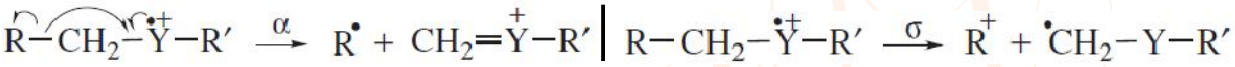
A radical-site-initiated fragmentation reaction is the cleavage of α—bond to the radical cation site by a transfer of the unpaired electron to form a *new bond to an adjacent atom* (α atom) with concomitant cleavage of another bond of this atom.

Competitive homolytic dissociation of asymmetric molecules depends on the delocalization of lone pair of adjacent heteroatom. The fragment obtained from the dissociation of the bigger radical has a higher intensity is preferred

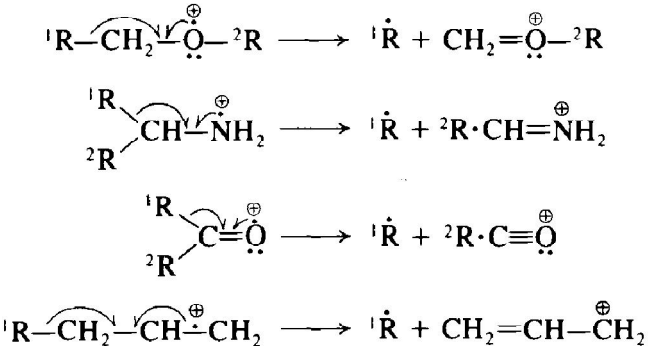
since the bigger radical can absorb more vibrational and rotational energy than that of a smaller radical from dissociation.



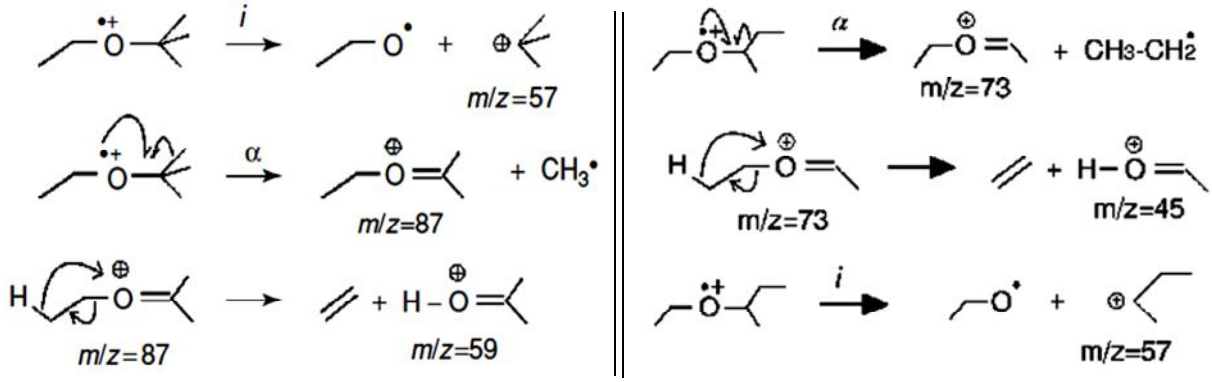
By Stevenson's rule, α—cleavage could involve radical retention and charge migration if the ionization energy of $^{\bullet}CH_2YR'$ is more than of $R^{\bullet}$.



Examples for α-cleavage



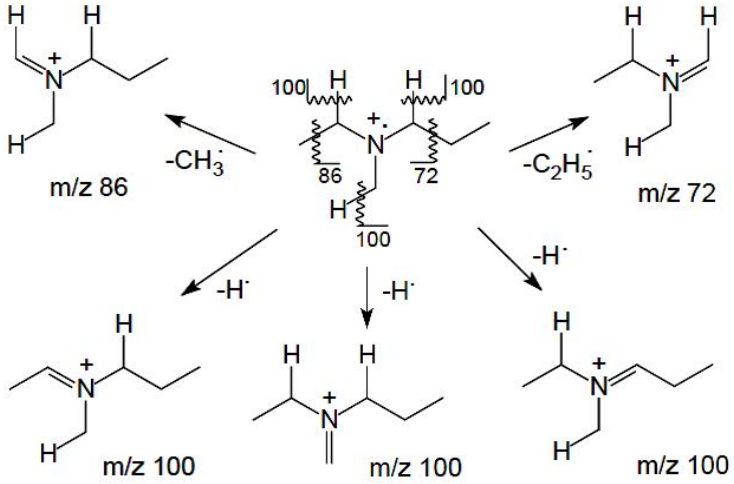
Fragmentations of t-butyl ethyl ether (left) and ethyl 2-butyl ether (right) are shown below.



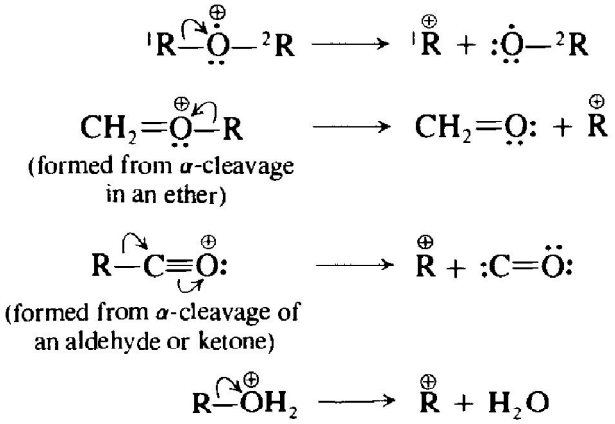
The cleavage of the adjacent bond occurs more easily if the heteroatom is a large atom. In the case of neighbouring atoms, the more electronegative one leads more easily to the cleavage of the adjacent bond. The α—cleavage becomes predominant for electron donors.

$$Br,\ Cl < R^{\bullet},\ \pi\,bond,\ S,\ O < N$$

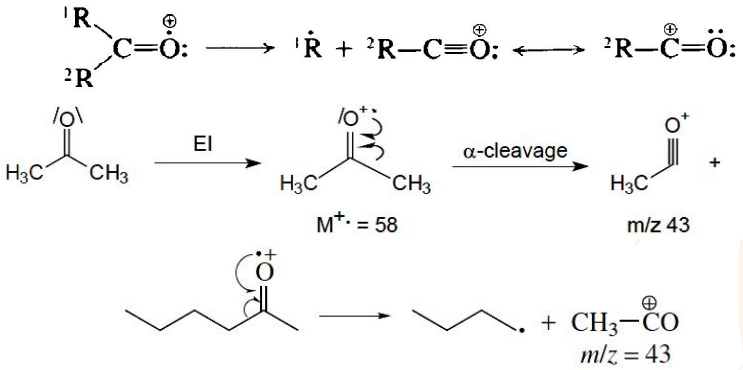
Ethyl-methyl-propylamine molecular ions undergo five different alpha-cleavages. Three of them results in isobaric product ions.



<u>Ex #6:</u> Fragmentation by movement of electron pair



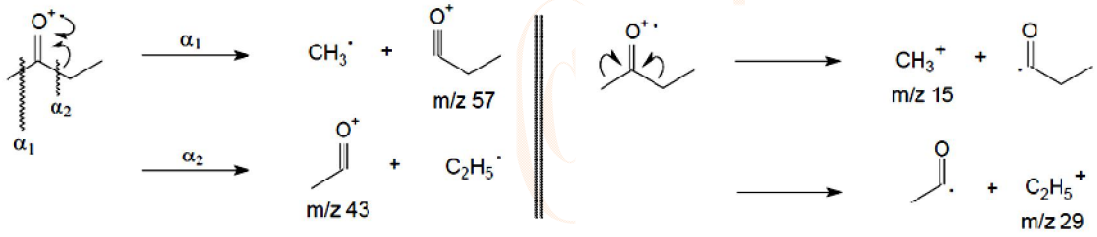
Ex #7: α—cleavage by homolytic dissociation with loss of alkyl radical and resonance stabilized acylium ion.



The molecular ion is formed by removal from the molecule of the electron of lowest ionisation potential. The energy required to remove an electron varies in the order

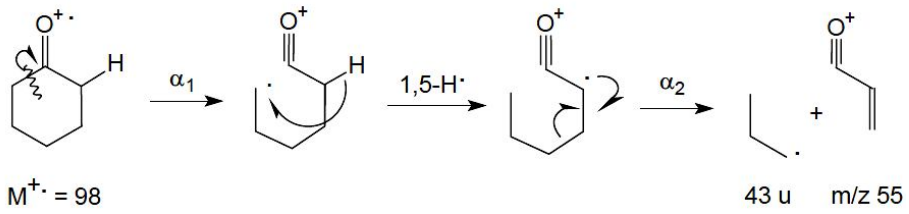
lone-pair < conjugated π < non-conjugated π < σ

The cleavage of 2-butanone is shown below.

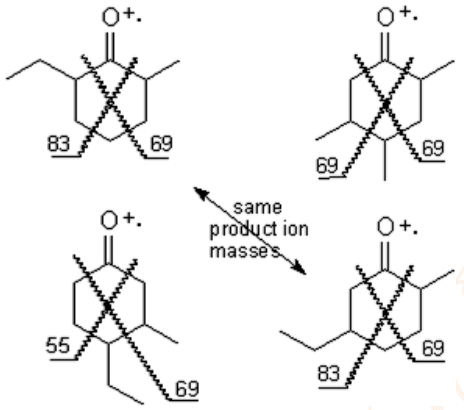


 # *Double* α—cleavage

The double α—cleavage of cyclohexanone is associated with loss of propyl group [M−43]+, m/z 55, from the molecular ion, M+• = 98. It was confirmed by deuterium-labeling to *both* α—positions of cyclohexanone. A three-step mechanism for propyl loss, via a double α—cleavage and an intermediate 1,5-H• shift are consistent with the experimental findings.

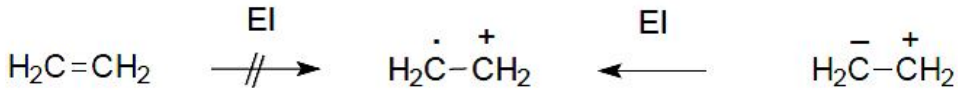


Four isomeric alkylcyclohexanone molecular ions are distinguished based on double-alpha cleavage.

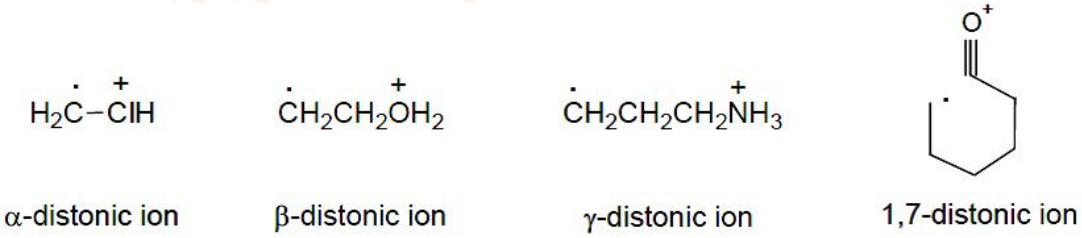


Distonic Ions

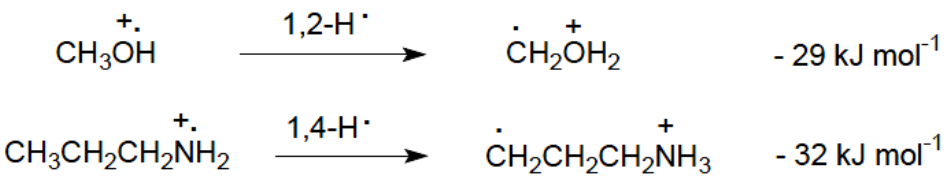
A distonic ion is characterized by a positive radical ion, which would formally arise by ionization of a zwitterion or a diradical, by isomerization or fragmentation of a classical molecular ion, or by ion-molecule reactions.



The examples of the terms α- (1,2-) distonic ion, β- (1,3-) distonic ion, γ- (1,4-) distonic ion distonic ions are listed below.

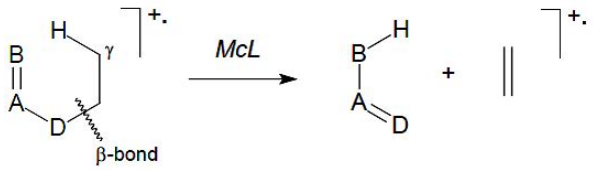


The distonic isomers are often thermodynamically more stable than their "classical" counterparts.

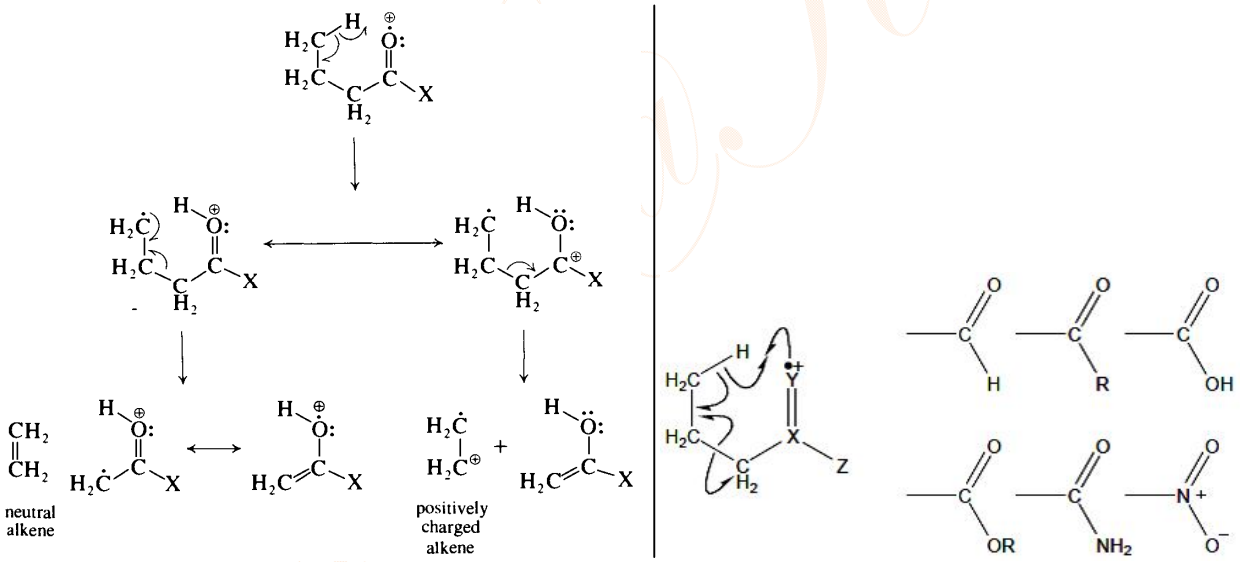


Ex #8: McLafferty Rearrangement: A γ-hydrogen abstraction

A transfer of a γ-hydrogen to a double-bonded atom through a six membered T.S with β-bond cleavage is regarded as McLafferty rearrangement (McL).



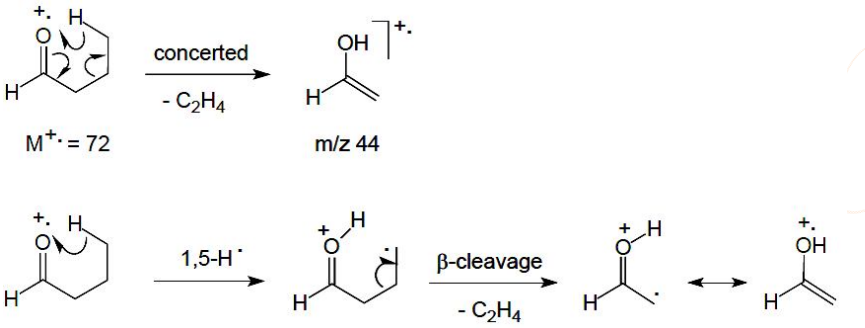
The mechanism involves in the abstraction of the γ—H atom by carbonyl oxygen atom followed by elimination of alkene through homolytic cleavage of the C_α-C_β bond. The mechanism is analogous to the Norrish type-II photo-fragmentation in condensed-phase chemistry.

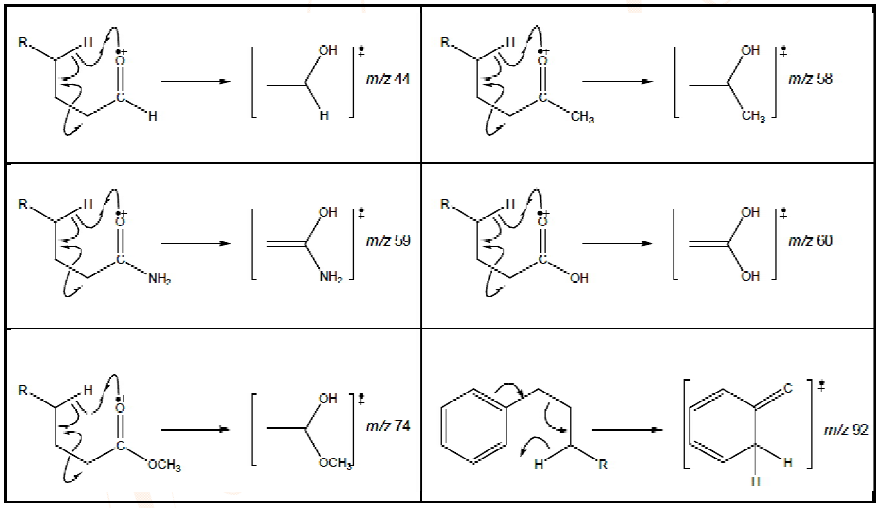


In order to have McL, the distance between the γ-hydrogen and the double-bonded atom must be less than 1.8 Å and the C_γ–H bond must be in plane with the acceptor group.

Is the McLafferty rearrangement concerted or stepwise?

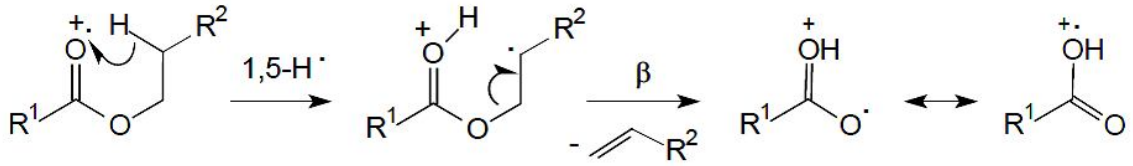
It remains elusive whether the reaction follows either concerted or stepwise pathway. The concerted pathway has been preferred in early publications. However, evidence for a stepwise mechanism involving distonic ion intermediates is presented in more recent work taking kinetic isotope effects into account.



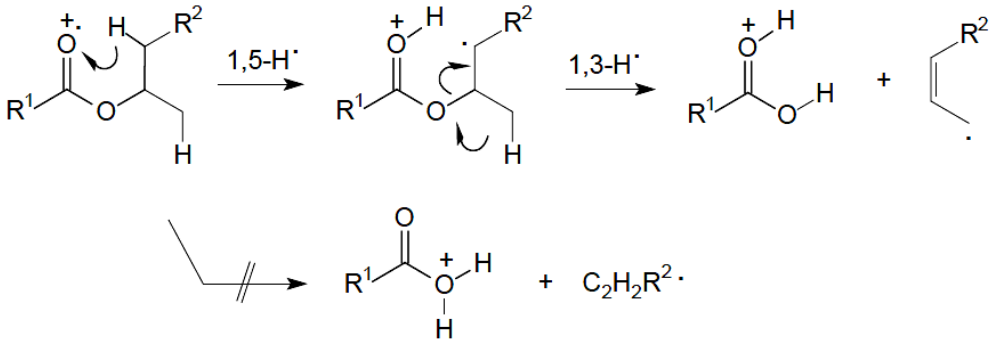


The role of the ϒ—hydrogen can only be understood after blocking the ϒ-position by introduction of alkyl or halogen substituents, that effectively hinders this dissociation pathway. For example, the absence of ϒ—hydrogen of 3,3-dimethyl-2-butanone rules out McL pathway.

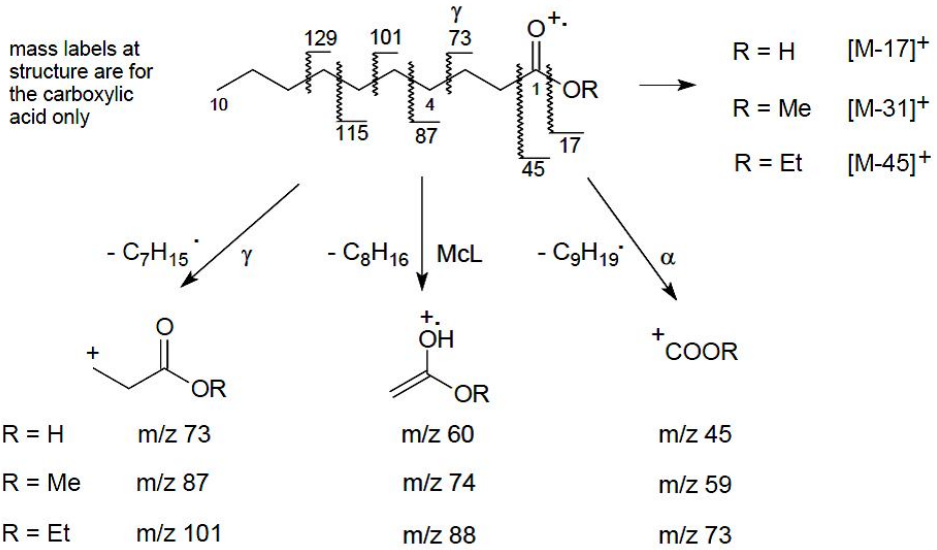
For carboxylic acid with longer aliphatic chain esters, the McL occurs on the alkoxy branch (R^2) of the molecular ion.

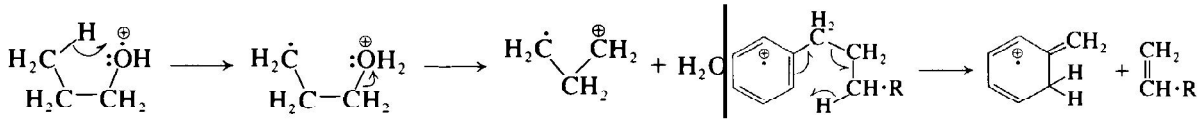


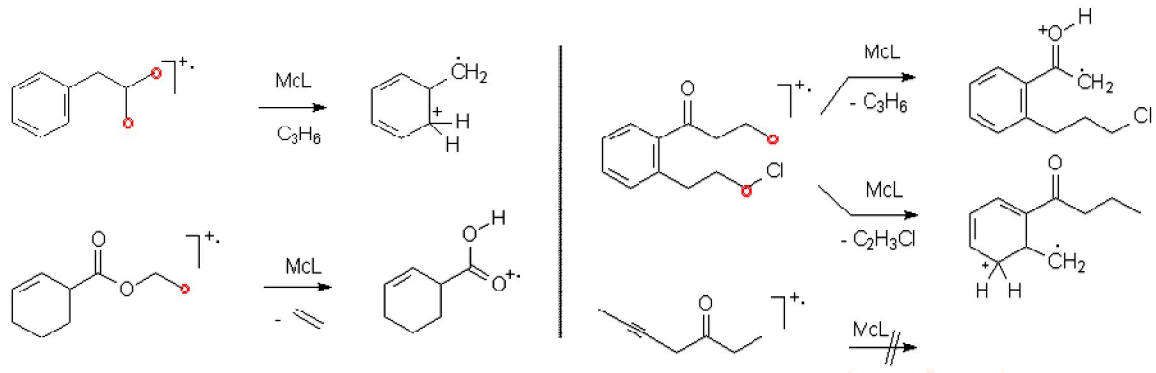
If there are two γ—Hs, the fragmentation follows the stability of radical after internal H-abstraction.



Fragmentation of carboxylic acid

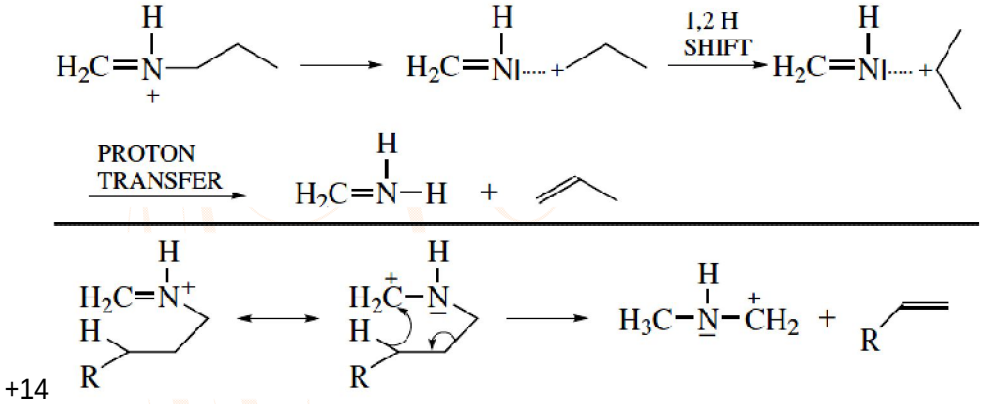






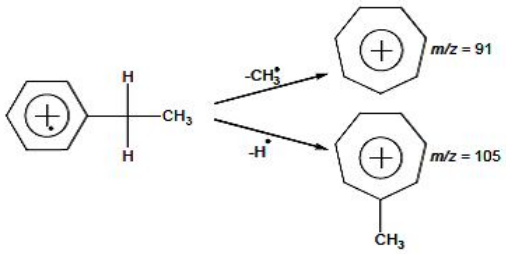
α or ϒ Cleavage of iminium ion

The McL of onium ions is accompanied by the same 100 % regioselectivity for ϒ—H transfer as is observed for odd-electron ions. Iminium ions with short alkyl chains fragment by forming an ion–neutral complex through 1,2 proton transfer whereas McLafferty rearrangement occurs with longer alkyl chain via 1,5 shift.

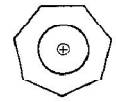


Ex #9: Formation of resonance stabilized stable carbocation; a seven membered stable tropylium cation $C_7H_7^+$ as thermodynamically most stable isomer.

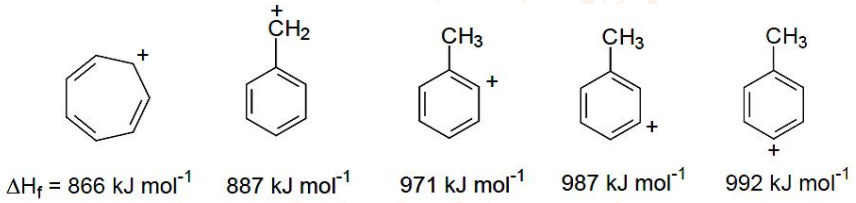
Molecular ions of phenylalkanes are comparatively stable and showing intense peaks due to good charge stabilizing properties of the aromatic ring.



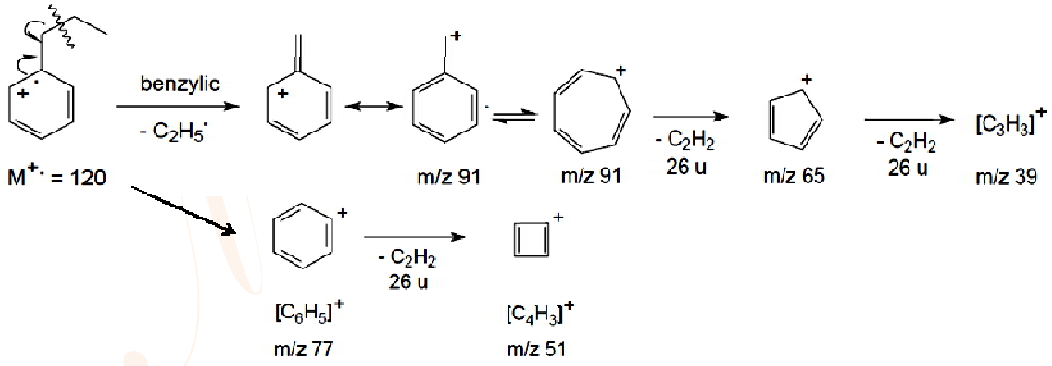
$$C_6H_5{\cdot}CH_2{-}R]^{\oplus\bullet} \longrightarrow \dot{R} +$$



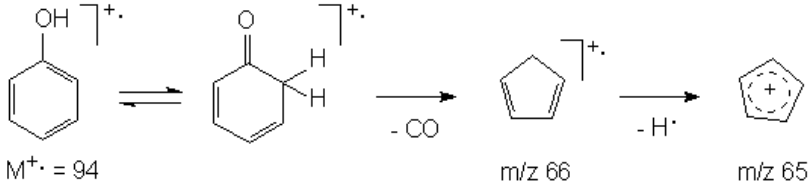
The benzyl—tropylium isomerization might be reversible (E_0 = 167 kJ mol^{-1}) as observed from the phenomenon of scrambling of hydrogens. However, benzyl-totropylium ratio depends on the structure of the precursor molecular ion. Thus, in 70 eV EI, $[C_7H_7]^+$ ions derived from benzyl chloride yield benzyl-totropylium ratio 57 : 43 whereas in 12 eV EI, the ratio becomes 92 : 8.



The EI mass spectra of phenylalkanes reveal the ion series m/z 39, 51, 65, 77, 91 and molecular ion in which m/z corresponds to 91 is most intensed.



<u>Ex #10:</u> Cleavage of phenol compounds



Rule of thumb:

The stability of molecular ions roughly decreases in the following order: aromatic compounds > conjugated alkenes > alkenes > alicyclic compounds > carbonyl compounds > linear alkanes > ethers > esters > amines > carboxylic acids > alcohols > branched alkanes.

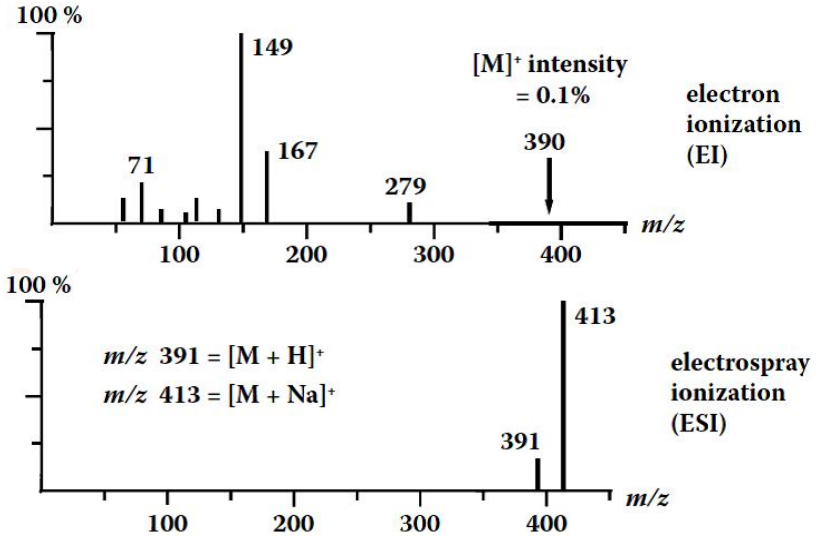
The stability of the molecular ion increases if π—bonding electrons for the delocalization of the charge are available and it decreases in the presence of preferred sites for bond cleavage, e.g., by α—cleavage.

Electrospray ionization (ESI) MS

Electrospray ionization (ESI) is a **soft ionization technique** that enables the transfer of ions (of polar compounds) from solution to the gas phase without causing decomposition. It is typically used to determine the molecular weights of large, nonvolatile, chargeable molecules such as peptides, proteins, nucleic acid polymers and other macromolecules.

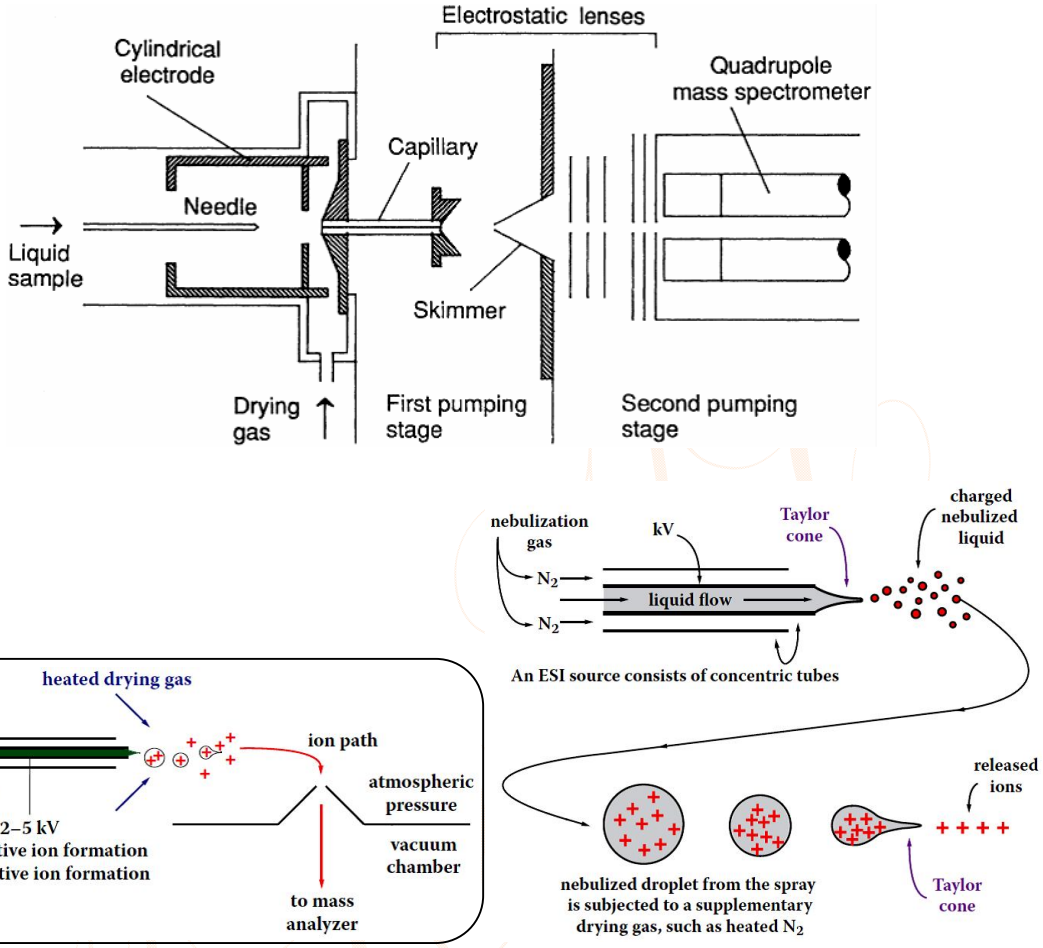
Electrospray ionization mass spectrometry is a **desorption ionization method.** It is performed on solid or liquid samples (analyte) which produce ions in a charged, nebulized solution of the analyte. This process **does not fragment** the macromolecules into smaller charged particles rather macromolecule being ionized into small droplets. These droplets are then desolvated (dried) and disintegrated into even smaller droplets, which creates molecular ions as attached protons in gas phase. These protonated and desolvated **molecular ions** are then passed through the mass analyzer to the detector, and the mass of the sample is determined.

The difference in ionization of dioctylphthalate in EI and ESI is shown below.



INSTRUMENTAL METHODS OF CHEMICAL ANALYSIS
Mass Spectrometry

Ionization mechanism



The events of the process are summarized below:

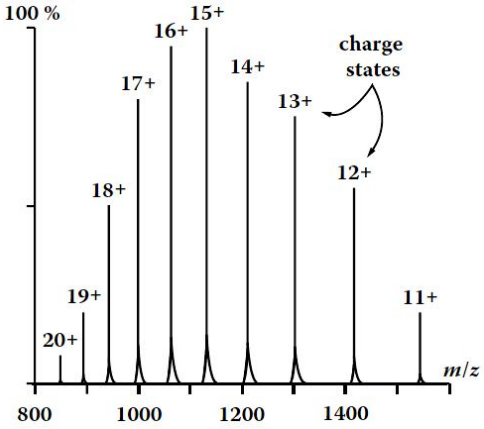
■ An analyte is dissolved in a solution (electrically conductive) of volatile organic solvent (acetonitrile : water or methanol : water) containing an organic acid (low pH usually 0.1% acetic acid or formic acid to decrease the initial droplet size of compounds to increase conductivity and provides a source of protons to facilitate the ionization process) flows through a narrow steel tube subjected to **high voltage potential 2–5 kV** (positive or negative) in vacuum.

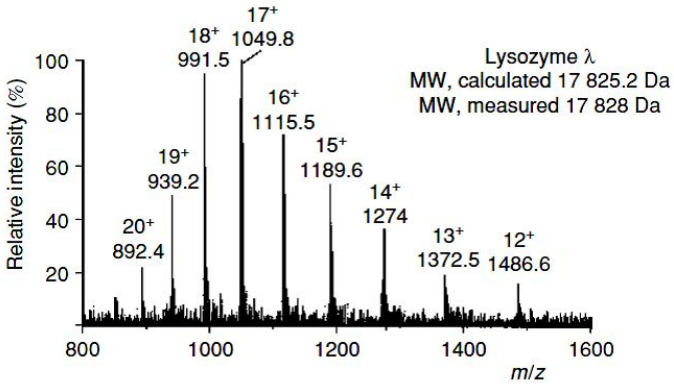
- The voltage removes ions of polarity opposite to that of the applied voltage, yielding a solution where there is only one charge type.
- A sheath gas usually nitrogen flowing (~7 bar, 100 psi) through outer tube (carrying the liquid), nebulizes the eluate into a spray of **charged droplets** that sprayed out at the end of the capillary (ESI probe) into the heated chamber to create an aerosol at nearly atmospheric pressure.
- A Taylor cone is developed at the tip of the ESI probe where the droplets are formed as the solvent flow is nebulized. Because it results in a stable stream of droplets from which the ions are released.
- The charge droplets are released into the ionization chamber. The charge droplets are then subjected to a counterflow of a drying gas (usually nitrogen) that evaporates solvent molecule from the droplets.
- The charge density of the droplet increases upon drying. The original charge droplets usually disintegrate into many smaller droplets (Coulomb and jet fission) when the electrostatic (Coulombic) repulsive forces exceeds the surface tension of the droplets (the Rayleigh limit). The process continues until **solvent-free sample charged ions** are left in gas phase.
- Continuous evaporation of droplets causes distortion and forms extension with concave surfaces, called a **Taylor cone** from which ions are released.

Notes

☑ The MS by ESI mode are dominated by molecular species without fragment because of acquiring limited amount of energy by analytes. No active chemical process is involved, such as bombardment with electrons or reaction with a proton donating species.

- ☑ ESI is highly dependent on the polarity of the analyte to form ions by taking up a proton or alkali metal ion from solution. For instance, nonpolar or lipophilic steroid with a single hydroxyl group usually give no or very poor ESI response while a natural product containing multiple amines will ionize readily.
- ☑ In positive ESI, ions are formed from neutral analytes at low pH by protonation $[M + H]^+$ or other cation attachment $[M + Na]^+$, $[M + K]^+$, and $[M + NH_4]^+$. In negative ESI, deprotonation $[M - H]^-$ or anion attachment $[M + Cl]^-$ or $[M + CH_3COOH]^-$ occurs at well above a molecules isoelectric point.
- ☑ ESI has the propensity to form multiply charged ions, i.e., $[M + nH]^{n+}$ for large molecules since it consist with multiple sites where number of charges can be added. It forms **envelop of ions with multiple charge states.** For instance, a peptide of MW 2,000 Da does not necessarily demonstrate an ion at m/z = 2,001 corresponding to $[M + H]^+$, rather exhibits ions at m/z = 1,001 $[M + 2H]^{2+}$, m/z = 667.7 $[M + 3H]^{3+}$, m/z = 501 $[M + 4H]^{4+}$. **All these ions are molecular species and are equally valid representations of the peptide**. The spectra of apo-myoglobin (left) and lysozyme λ (right) show ions in the +20 to +11 charge state range, between m/z 848 and 1,542 and m/z 892 and 1486 respectively.





☑ The solution of a set of simultaneous equations derived from the differences between the charge states, called **deconvolution**, yields the molecular mass of the protein or other biopolymers.

☑ Charges of ions from ESI do not reflect the charge state of compounds in the analysed solution; rather charge accumulation in the droplets. Typically, a protein will carry one charge per thousand Daltons. Thus, for a 10 kDa protein with charge states of 15, 14, 13, 12, 11, and 10, ions can demonstrate at m/z 668, 715, 770, 834, 910, and 1,001 respectively, when the masses of the added protons are included. Therefore, the spacing between the ions will be 47, 55, 64, 76, and 91 Da, i.e there there is progressively increasing spacing between the multiply charged ions in the spectrum indicating a biopolymer, most often a protein.

Advantages

- A soft ionization method to analyze large masses including biological samples that are defined by non-covalent interactions.
- Proteins can be ionized without denaturization and also in the form of noncovalent, receptor covalent, receptor-ligand complexes.
- Working directly from a dilute solution.
- Any polar solvent (H_2O, ACN, THF etc) suitable

Disadvantages

- This technique can't analyze mixtures very well, and sometimes unreliable.
- The apparatus requires difficult for cleaning and has a tendency to become overly contaminated with residues from previous experiments.
- Multiple charges to molecular ions sometimes confuse spectral data.

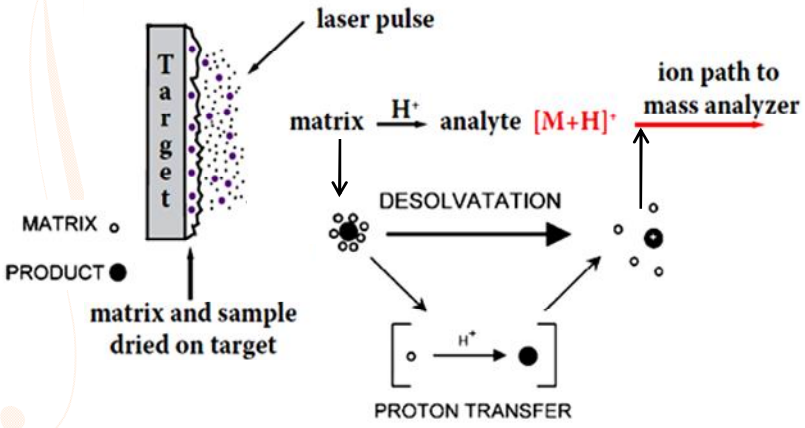
Matrix-Assisted Laser Desorption Ionization (MALDI)

MALDI is a soft ionization technique that uses laser to ionize the molecule directly by producing intact gas-phase ions from non-volatile and thermally labile large MW compounds. It analyzes biomacromolecules (biopolymers such as proteins, peptides, oligonucleotides and sugars) and large organic molecules (such as synthetic polymers, dendrimers).

Laser: UV lasers are commonly used because of ease of operation and low price. N_2 lasers (λ = 337 nm) are considered as the standard, though Nd:YAG lasers (λ = 266 or 355 nm) are also used. MALDI can also use IR lasers like Er:YAG lasers (λ = 2.94 µm) or CO_2 lasers (λ = 10.6 µm).

Ionization method: Use of matrix that provides desorption and ionization.

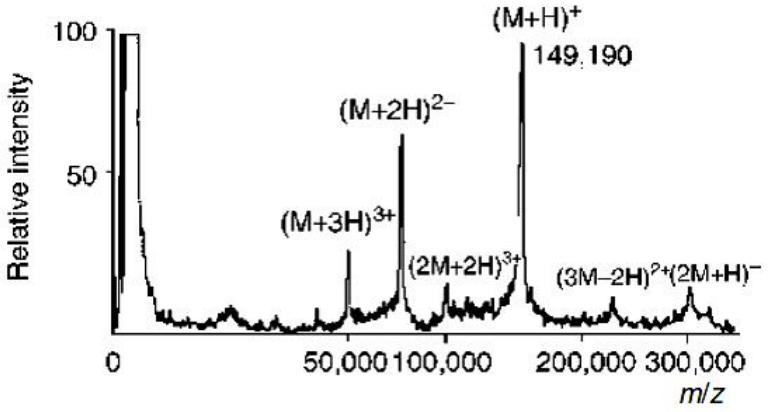
Mechanism of ionization: MALDI produces only molecular ion peak. The use of a matrix (usually an aromatic acid) in excess molar proportion over the analyte does not allow fragmentation since the matrix absorbs most of the energy from laser. The reason is described below.



The laser is fired at the matrix crystals in the dried droplet spot placed in vacuum. The matrix absorbs the pulsed (UV) laser energy and get excited. The laser causes desorption of matrix and sample from the microcrystaline surface to vapour phase. The excited matrix molecules adhere proton

forming protonated matrix ions. The excited matrix ions transfer protons to the analyte molecules (e.g., protein, nucleic acid etc.), thus charging the analyte molecules to quasimolecular ions $[M+H]^+$ or $[M+Na]^+$ or $[M-H]^-$ in vapour phase. So, basically the matrix absorbs the energy from the laser pulse and produces a plasma that results in vaporization and ionization of the analyte. The speed of the process reduces the probability of degrading the analyte.

MALDI spectra of a monoclonal antibody is shown below.



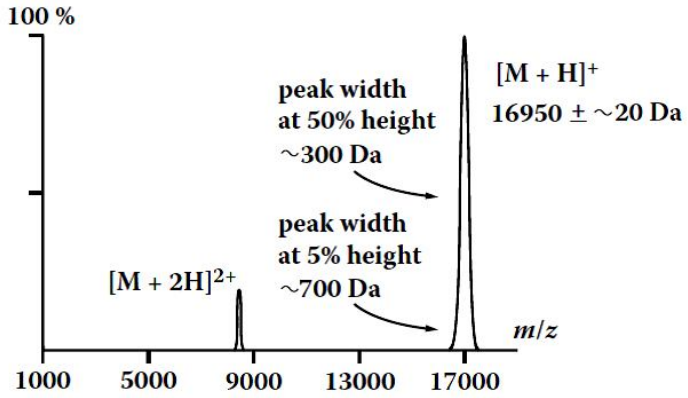
Q. Why are 2,5 DHBA, CHCA, Sinapinic acid used as common UV-MALDI matrices?

Ans: Samples and matrices are prepared in an acetonitrile:water (1:1 v/v) mixture containing 0.1–1% trifluoroacetic acid. The concentration of the matrix is maintained ~10 mg/ml. These matrices are nonvolatile, relatively inert, reasonable electrolyte to allow ion formation. Since they are acids and if their acidity is more than the analyte, the **$[(M + H)^+]$** ions will be formed and (**$[(M - H)^+]$** is produced if matrices are less acidic than sample. Further, these matrices have their ability to absorb UV light from a laser pulse (337 nm for N_2 laser). The matrix absorbs most of the energy from the laser

pulse and thus allows the creation of intact sample ions that are ejected from the matrix.

The matrix minimizes the damage of the sample from the laser pulse by absorbing most of the incident energy and increases the efficiency of energy transfer from the laser to the analyte. The 2,5 dihydroxybenzoic acid (DHBA), α-cyano-4-hydroxy-cinnamic acid (CHCA) are useful for MW upto 5kDa while sinapinic acid is used for larger MW of peptides and proteins. Because CHCA transfers larger amounts of energy to analytes than does sinapinic acid, causing in the destruction of larger MW of biopolymers.

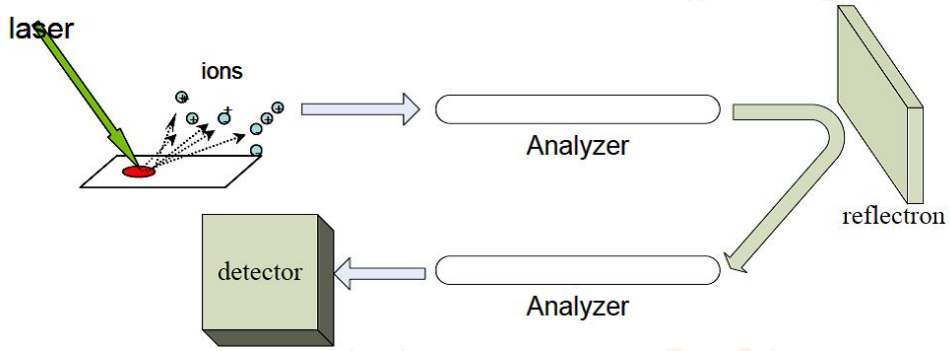
MALDI spectra are composed predominantly of singly charged ions, although double and triple charge states may be seen when analyzing species with larger molecular mass, as illustrated by the low intensity $[M + 2H]^{2+}$ ion of myoglobin.



The Time of flight (TOF) is a method for measuring particle mass-to-charge ratio. An ion of known electrical charge and unknown mass enters a mass spectrometer and is accelerated by an electrical field of known strength. This acceleration results in any given ion having the same kinetic energy as any other ion given that they all have the same charge. The velocity of the

ion will depend however on the mass-to-charge ratio. Thus, MALDI deals with the ionization of the sample in the source, while TOF discusses about the ions in the analyzer.

The TOF mass analyzer measures the time it takes for the ions to fly from one end of the analyzer to the other and strike the detector. The flying speeds of ions are proportional to their mass-to-charge ratio.



The workflow in reflectron mode is much similar as in linear mode. The only difference is that when the ion hit the reflectron, it will reflect and fly towards the detector. The reflectron focuses ions with the same m/z values, and makes them reach the detector at the same time, which results in more accurate detection.

Benefits

- Rapid and convenient molecular weight determination
- Mass range very high typically less than 500,000 Da

Limitations

- MS/MS difficult
- Requires a mass analyzer that is compatible with pulsed ionization techniques
- Not easily compatible with LC/MS

References

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