CHAPTER: 1

INTRODUCTION
1.1. Introduction to Organic Mass Spectrometry

1.1.1. The Mass Spectrometer

A mass spectrometer can be used to determine the molecular mass, molecular formula and information regarding the structure of an organic molecule. In order to study the characteristics of individual molecules, a mass spectrometer converts them to ions so that they can be accelerated and separated by external electric and magnetic fields. The three essential components of a mass spectrometer \[1a-c\] and their associated functions are:

i. The Ion Source: Here the compound to be analyzed is ionized, usually to cations by loss of an electron or by protonation.

ii. The Mass Analyzer: Here the ions are sorted and separated according to their mass to charge ratios.

iii. The Ion Detector: The separated ions are then detected, tallied and the results are displayed on a chart.

Since ions are very reactive and short-lived, their formation and manipulation must be conducted in vacuum. The pressure under which ions may be handled is around \(10^{-7}\) torr. Each of the three tasks listed above may be accomplished in different ways. In one common procedure (EI), a high-energy beam of electrons effect the ionization and ion separation is achieved by accelerating and focusing the ions in a beam, which is then deflected by an external magnetic field. The ions are then detected electronically and the resulting information is stored and analyzed by using a computer. A mass spectrometer operating in this fashion is outlined in Fig.1.1.
When a high-energy electron collides with a molecule, it is often ionized by knocking away one of the electrons of the molecule and leaves behind a molecular ion. Residual energy from the collision may cause the molecular ion to fragment into neutral species and smaller fragment ions, Scheme 1.1. The molecular ion is always a radical cation, but the fragment ions may either be radical cations or cations depending on the nature of the neutral fragment.
1.1.2. The Nature of Mass Spectra

A mass spectrum will usually be presented as a vertical bar graph, in which each bar represents an ion having a specific mass-to-charge ratio ($m/z$) and the length of the bar indicates the relative abundance of the ion. The most intense ion is assigned an abundance of 100, and it is referred to as the base peak [1b]. Most of the ions formed in a mass spectrometer have a single charge, so the $m/z$ value is equivalent to mass itself. Modern mass spectrometers can measure the masses with an accuracy of up to $10^{-4}$ amu and thus provide the molecular formula of a compound.

Most of the organic molecules have an even number of total electrons. When a single electron is removed from a molecule to give an ion, the total electron count becomes an odd number, and we refer to such ions as radical cations [1a-c]. The simplest and most common fragmentations are bond cleavages producing a neutral radical (odd number of electrons) and a cation having an even number of electrons. A less common fragmentation, in which an even-electron neutral fragment is lost, produces an odd-electron radical cation fragment ion. Fragment ions themselves may fragment further. As a rule, odd-electron ions may fragment either to odd or even-electron ions, but even-electron ions fragment only to other even-electron ions. [1a-c]

1.2. Ionization Methods in Organic Mass Spectrometry

1.2.1. Introduction

A Mass Spectrometer works by using magnetic and electric fields to exert forces on charged particles (ions) in a vacuum to separate them. Therefore, a molecule must be charged or ionized before analyzed by a mass spectrometer in the gas-phase. This is easily done for gaseous or volatile samples. However, thermally labile analytes decompose upon heating.
These kinds of samples require either desorption or desolvation methods if they are to be analyzed by mass spectrometry. Although ionization and desorption or desolvation are usually separate processes, the term "ionization method" [1a] is commonly used to refer to both ionization and desorption (or desolvation) methods. The choice of ionization method depends on the nature of the sample and the type of information required from the analysis. So-called 'soft ionization' [1a] methods such as Laser desorption and electrospray ionization tend to produce mass spectra with little or no fragment ions.

1.2.2. Gas-Phase Ionization

These methods rely upon ionizing gas-phase samples. The samples are usually introduced through a heated inlet for liquids, heated direct insertion probe for solids, or a gas chromatograph.

a. Electron Ionization (EI)

This is the oldest of all the ionization methods and is also referred to as electron impact ionization [1a-c, 2]. A beam of high–energy electrons passes through the sample in the gas-phase. An electron that collides with a neutral analyte molecule can knock off another electron, resulting in a positively charged ion. The ionization process can either produce a molecular ion, which will have the same molecular weight and elemental composition of the starting analyte, or it can produce fragment ions, which correspond to a smaller piece of the analyte molecule. The ionization potential is the energy that will produce a molecular ion. The appearance potential for a given fragment ion is the electron energy that will produce that ion. Most mass spectrometers use electrons with energy of 70 electron volts (eV) for EI. Decreasing the electron energy reduces fragmentations, but reduces the number of ions.
b. Chemical Ionization (CI)

Chemical ionization [1a-c] uses ion-molecule reactions to produce ions from the analyte. The chemical ionization process begins when a reagent gas such as methane, isobutane, or ammonia is ionized by electron impact. A high reagent gas pressure or long reaction time results in ion-molecule reactions between the reagent gas ions and reagent gas neutrals. Some of the products of these ion-molecule reactions can react with the analyte molecules to produce protonated analyte molecules.

\[
R + e^- \rightarrow R^+ + 2e^- \\
R^+ + RH \rightarrow RH^+ + R' \\
RH^+ + S \rightarrow SH^+ + R
\]

(R = reagent gas, S = sample, e = electron)

This often gives molecular weight information through molecular-like ions such as protonated molecule \([M+H]^+\), even when electron impact would not produce a molecular ion.

1.2.3. Particle Bombardment

In these methods, the sample is deposited on a target that is bombarded with atoms, neutrals, or ions. The most common approach for organic mass spectrometry is to dissolve the analyte in a liquid matrix with low volatility and to use a relatively high current of bombarding particles [1a-c, 2,3] (FAB or dynamic SIMS). Other methods use a relatively low current of bombarding particles and no liquid matrix (static SIMS). The latter methods are more commonly used for surface analysis than for inorganic mass spectrometry. The primary particle beam is the bombarding particle beam, while the secondary ions are the ions produced from bombardment of the target.
a. Fast Atom Bombardment (FAB)

The analyte is dissolved in a liquid matrix such as glycerol, thioglycerol, m-nitrobenzyl alcohol, or diethanolamine. A small amount of a solution of the analyte (about 1 microlitre) is placed on a target [4a]. The target is bombarded with a fast atom beam (for example, 6 KeV, Argon atoms) that desorbs as protonated molecule, \([\text{M+H}]^{+}\) ions and fragments from the analyte. Cluster ions from the liquid matrix are also desorbed and produce a chemical background that varies with the matrix used.

b. Secondary Ion Mass Spectrometry (Liquid SIMS)

Liquid or dynamic SIMS is nearly identical to FAB except that the primary particle beam is an ion beam usually cesium ions rather than a neutral beam. The ions can be focused and accelerated to higher kinetic energies than are possible for neutral beams, and sensitivity is improved for higher masses.

1.2.4. Electrospray Ionization (ESI)

In this method, [1a-c, 6b-e] a solution of the analyte in a suitable solvent such as 1:1 mixture of acetonitrile and water is sprayed across a high potential difference (~ 3 kilovolts) from a needle into the interface at the atmospheric pressure. Heat and nitrogen gas flows are used to desolvate the ions existing in the sample solution before they enter the ion source. Electrospray ionization can produce singly or multiply protonated analyte molecules with low internal energy. Solution flow rates can range from less than a microlitre per minute to several micro liters per minute. These methods are well suited for flow-injection and LC/MS techniques. High molecular weight molecules such as proteins, lipids or polymers can be analysed by this technique.
1.2.5. Matrix-Assisted Laser Desorption Ionization (MALDI)

Laser desorption methods use a pulsed laser to desorb species from a target surface. Therefore, one must use a mass analyzer such as time-of-flight (TOF) [1c, 5a-c] that is compatible with pulsed ionization methods. Direct laser desorption relies on the very rapid heating of the sample or sample substrate to vaporize molecules so quickly that they do not have time to decompose. This is good for low to medium-molecular weight compounds and surface analysis. The more recent development of matrix-assisted laser desorption ionization (MALDI) [4] relies on the absorption of laser energy by a matrix compound. MALDI has become extremely popular as a method for the rapid determination of high-molecular-weight compounds and peptides.

The analyte is dissolved in a solution containing an excess of a matrix such as 4-hydroxy α-cyano cinnamic acid, sinapinic acid or dihydroxybenzoic acid that has a chromophore that absorbs at the laser wavelength. A small amount of this solution is placed on the laser target and the solvent is allowed to evaporate. The solid matrix absorbs the energy from the laser pulse and protonate the analyte molecule.

In the present work we have used EI, CI, FAB and ESI for the mass spectral analysis of the molecules.

1.3. Mass Analysis

Immediately following ionization in gas phase, the ions enter a region of the mass spectrometer known as the mass analyzer. The mass analyzer is used to separate ions within a selected range of mass-to-charge (m/z) ratios [1a-c]. The analyzer is an important part of the instrument because of the role it plays in the instrument's accuracy and mass range. Ions are typically separated by magnetic fields, electric fields, or by measuring the time it takes an ion
to travel a fixed distance. The mass spectrometer mass scale is calibrated, with the aid of the data system, by introducing a reference compound that yields a well characterized mass spectrum containing known masses at suitable intervals, (for example, perfluorokerosene is used for calibrating the mass scale in EI mode). This allows the time and intensity information to be converted to mass to charge ratios and ion abundances.

1.3.1. Sector instruments

Double focusing mass spectrometers [4a,b] utilize a combination of magnetic sector and electric sector to separate ions according to their momentum to charge ratios. The earliest mass spectrometers separated ions with a magnetic field alone. In magnetic analysis, the ions are accelerated and are passed into a magnetic field. A charged particle traveling at high speed through a magnetic field will experience a force, and travel in a circular path with a radius depending upon the \( m/z \) and speed of the ion. A primary limitation of typical magnetic analyzers is their relatively low resolution. In order to improve resolution, single-sector magnetic instruments have been replaced with double-sector instruments by combining the magnetic mass analyzer with an electrostatic analyzer. The electric sector acts as a kinetic energy filter allowing only ions of a particular kinetic energy to pass through, irrespective of their mass-to-charge ratios.

The radius of curvature, \( r \) for the fragment ion is \( r = 2V/E \), where \( E \) is the field, applied between two curved plates and \( V \) the energy. Thus, the addition of an electric sector allows only ions of uniform kinetic energy to reach the detector, thereby increasing the resolution of the two-sector instrument to 50,000. Magnetic double-focusing instrumentation is commonly used with FAB and EI ionization however they are not used for ESI and MALDI ionization primarily because of the pressure in the ion source and mass range.
1.3.2. Quadrupole mass analysers [1a-c]

Quadrupoles are four precisely parallel rods with a direct current (DC) voltage and a superimposed radio-frequency (RF) potential. By scanning a pre-selected radio frequency field, one effectively scans a mass range. Quadrupole mass analyzers [1a-c, 6a-e] have been used in conjunction with electron ionization sources since the 1950s. Quadrupoles have three primary advantages. First, they are tolerant of relatively poor vacuums (~ 5 x 10^{-5} torr), which make them well suited to electrospray ionization since the ions are produced under atmospheric pressure conditions. Secondly, quadrupoles are now capable of routinely analyzing up to \( m/z \) of 2000, which is useful because electrospray ionization of proteins and other biomolecules commonly produces a charge distribution below \( m/z \) 2000. Finally, the relatively low cost of quadrupole mass spectrometers makes them attractive. Considering these mutually beneficial features of electrospray and quadrupoles, it is not surprising that most of the successful commercial electrospray instruments thus far have been coupled with quadrupole mass analyzers.

1.3.3. Time-of-Flight

A time-of-flight (TOF) analyzer [1, 4a,b, 5a-c] is one of the simplest mass analyzing devices and is commonly used with MALDI and ESI. Time-of-flight analysis is based on accelerating a set of ions to a detector with the same amount of energy. These ions have to traverse a flight tube of length approximately 1M. The flight tube is usually a vacuum enclosure, free of electrical fields, between the ion source and the detector. It is sometimes referred to as a field free drift region. The ions, which are under study, exit the ion source and enter the linear flight tube with the proper velocity and direction to arrive at the detector.
Since the ions have the same energy and different masses, the ions reach the detector at different times. The smaller ions reach the detector first because of their greater velocity and the larger ions take longer, thus the analyzer is called time-of-flight and the mass is determined by the time of arrival of the ions. The arrival time of an ion at the detector is dependent upon the mass, charge, and kinetic energy of the ion. Since kinetic energy (KE) is equal to \( \frac{1}{2} mv^2 \) or velocity \( v = (2KE/m)^{1/2} \), ions will travel a given distance, \( d \), within a time, \( t \), where \( t \) is dependent upon their \( m/z \). By using a reflectron the path length of the ions can be increased thereby increasing the resolution.

### 1.3.4. Ion Detectors

Once the ion passes through the mass analyzer the ion detector, [1a-c] the final element of the mass spectrometer, then detects it. The detector allows a mass spectrometer to generate a signal current from incident ions by generating secondary electrons, which are further amplified. Alternatively, some detectors operate by inducing a current generated by a moving charge. Among the detectors described, the electron multiplier and photon multiplier (scintillation counter) are the most commonly used and convert the kinetic energy of incident ions into a cascade of secondary electrons.

#### a. Electron Multiplier

An electron multiplier [1a-c] is one of the most common means of detecting ions, with high sensitivity working on the basic principle that a change in charge on a metal plate results in a flow of electrons and therefore creates a current. One ion striking a dynode surface (a dynode is a secondary emitting material, usually BeO, GaP, or CsSb) induces several secondary electrons to be ejected and temporarily displaced. This temporary emission of electrons induces a current and provides for a small amplification of signal when an ion
strikes the dynode. An electron multiplier is made up of a series of dynodes maintained at ever increasing potentials. Ions strike the dynode surface, resulting in the emission of electrons. These secondary electrons are then attracted to the next dynode where more secondary electrons are generated, ultimately resulting in a cascade of electrons. Typical amplification or current gain of an electron multiplier is one million.

b. Photo Multiplier

The photo multiplier [1a-c] conversion dynode detector is similar to an electron multiplier where the ions initially strike a dynode, resulting in the emission of electrons. However, in the photomultiplier conversion dynode detector, electrons then strike a phosphorus screen. The phosphorus screen, much like the screen on a television set, releases photons once an electron strikes. These photons are then detected by a photo multiplier, which operates with a cascading action much like an electron multiplier. The primary advantage of the conversion dynode setup is that the photomultiplier tube is sealed in a vacuum (photons pass through sealed glass), unexposed to the internal environment of the mass spectrometer. Thus the possibility of contamination is removed. A five-year or greater lifetime is typical and, with sensitivity similar to electron multipliers, photomultiplier conversion dynode detectors are becoming more widely used in mass spectrometers.

1.4. Tandem Mass Spectrometry

Tandem mass spectrometry (MS/MS) is used to elucidate structural information about a compound as well as ion fragmentation mechanisms by fragmenting specific precursor ions inside the mass spectrometer and identifying the resulting fragment ions [4a, 5 a-d]. This information can then be put together to generate structural information regarding the intact
molecule. It also enables specific compounds to be detected in complex mixtures on account of their specific and characteristic fragmentation patterns. The study of the structure of metastable ions can also be done using a tandem mass spectrometer. Metastable ions are ions that survive long enough to leave the ion source but decompose before reaching the detector.

A tandem mass spectrometer \([5a-d]\) is a mass spectrometer that has more than one analyser, in practice usually two. The two analysers (MS\(_1\) and MS\(_2\)) are separated by a collision cell, into which an inert gas (e.g. helium, argon and xenon) can be admitted to collide with the selected precursor ions and bring about their fragmentation. The mass spectrum is obtained by scanning the analyser following the collision cell. When the collision cell contains a gas the spectrum obtained is called collision activated dissociation (CAD) mass spectrum \([5a-d]\). If the collision cell does not contain a gas the spectrum obtained is known as metastable ion (MI) mass spectrum. When a combination of magnetic and electric sectors is used as MS\(_1\), the mass selection can be done at high resolution so that interferences can be avoided. The use of a combination of magnetic and electric sectors as MS\(_2\), the product ion mass spectrum can be obtained with a resolution of up to 2000 while the use of a TOF analyser as MS\(_2\), provides a resolution of the order of 15000 so that accurate masses of the product ions can be determined.

The analysers can be of the same or of different types; the most common combinations are cited below.

<table>
<thead>
<tr>
<th>Mass selection (MS(_1))</th>
<th>Mass analysis (MS(_2))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnetic sector</td>
<td>Electric sector (MIKES)</td>
</tr>
<tr>
<td>Magnetic and electric sectors</td>
<td>Magnetic and Electric sectors (Linked scan)</td>
</tr>
<tr>
<td>Quadrupole</td>
<td>Quadrupole</td>
</tr>
<tr>
<td>Quadrupole</td>
<td>Time-of-flight</td>
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</tbody>
</table>
The ion optics of a four sector mass spectrometer is shown in Fig.1.2.

![Diagram of ion optics of a four-sector mass spectrometer](image)

**Fig.1.2.** Ion optics of a four-sector mass spectrometer

The basic modes of data acquisition for a tandem mass spectrometer are as follows:

**1.4.1. Product ion mass spectrum:**

The first analyser is used to select the specified ions arising from a particular compound, i.e. molecular ions or fragment ions. These chosen ions will pass through the collision cell and the fragment ions are analysed by scanning the second analyser. A metastable ion (MI) or a collision activated dissociation (CAD) mass spectrum is obtained depending upon gas is absent or present, respectively, in the collision cell. The MI mass spectrum, contains relatively few fragments, is useful for determining the fragmentation pathways requiring low activation energy. The CAD mass spectrum is particularly useful for
providing structural information concerning small organic molecules by comparison with that of an authentic standard as well as for generating peptide sequence information.

When an electric sector is used as MS$_2$ in an MS/MS experiment the product ion spectrum obtained is known as a MIKE spectrum [5a-d]. The product ion spectrum obtained is called linked scan mass spectrum if MS$_2$ is a combination of magnetic and electric sectors since both sectors are scanned keeping the ratio of the strength of the two fields a constant i.e. B/E a constant (where B and E are the strength of the magnetic and electric fields, respectively).

1.4.2. Precursor ion mass spectrum:

The first analyser allows the transmission of all sample ions, while the second analyser is set to transmit a specific fragment ion, which is generated by the dissociation of all the possible precursor ions [5d]. The mass spectrum obtained is useful for elucidation of fragmentation pathways.

1.4.3. Constant neutral mass spectrum:

This involves both analysers [4a, 5a-d] scanning across the whole $m/z$ range, but the two are offset so that the second analyser allows only those ions, which differ, by a certain number of mass units (equivalent to a neutral fragment) from the ions transmitted through the first analyser.

1.4.4. Collision Activated Dissociation (CAD) Spectrum.

When an ion having a high translational energy collides with neutral atoms or molecules, part of its energy is converted to internal energy of the ion, which can lead to its fragmentation. He or Ar gas is introduced to a collision chamber in the field free region [5a-d]. It can be noted that the number of fragment ions in a CAD spectrum is more when
compared to a MI spectrum. CAD spectrum is very useful in studying the structure of a selected precursor ion.

### 1.5 Basic Fragmentations of Ions in Mass spectrometry.

The gas-phase ionisation reactions in mass spectrometry can be viewed as another field of chemistry. These reactions have close similarities to pyrolytic, photolytic, radiolytic and high-energy reactions and rearrangements in condensed phase but the mechanisms of reactions in mass spectrometry may be different due to the absent of a solvent. Broad classes of mass spectrometric fragmentations include unimolecular ion decompositions and radical rearrangements [1a-c]. Rearrangements include McLafferty rearrangements [6a], Hydrogen rearrangement to a saturated heteroatom with adjacent cleavage, displacement reactions leading to cyclisation, charge cite rearrangements etc. [1a-c.]. Cyclisation reactions generally follow a mechanism, which involves proximity effects. There are several groups, which show proximity effects of which nitro group was very fascinating for researchers in this area. Nitro group shows “ortho effect” which is a specific example of general proximity effects [7a-c].

### 1.5.1 Ortho effects of nitro group in mass spectrometry.

The mass spectra of 2-nitro aromatic compounds usually show peaks due to the ortho interaction of nitro group with the side chain. The ability of the nitro group to interact with the side chain is due to the presence of two electron rich oxygen atoms and its bulk. Bursey and co-workers studied the proximity effects of nitro group involving hydrogen migrations [8] while Schwarz has discussed the redox reactions of ortho substituted nitrobenzene derivatives on electron impact [9].
Benoit and Holmes [10] have classified the fragmentations generated by EI into (1) Those that involve the transfer of a hydrogen radical from the ortho substituent to the nitro group followed by rearrangements which will result in the elimination of small neutral molecules or radicals, (2) Those which involve the elimination of nitro group followed by the migration of atoms or groups from the remaining adjacent substituent to the vacant ortho position and (3) those that involve the migration of the oxygen atom from the nitro group to the other parts of the molecular ion or fragment ion.

Beynon and co-workers [11] have reported an abundant [M – OH]+ ion in the EI mass spectrum of 2-nitroaniline. A mechanism involving the interaction of nitro group with the amine function has been proposed (Scheme 1.5.1). This fragment is totally absent in the mass spectra of its meta- and para-isomers.

\[
\begin{align*}
\text{NH}_2^- & \text{NO}_2^- \\
\downarrow & \text{OH}^- \\
\text{NH}_2^+ & \text{NO}_2^- \\
\end{align*}
\]

Scheme 1.5.1

Beynon and co-workers [12, 13] have noticed that in the mass spectrum of 2-nitrotoluene the ions corresponding to [M - OH]+, [(M – OH) - CO]+, [(M – OH - CO)-HCN]+, [(M – OH)- HCN]+ and [(M – OH) - NO]+ are the prominent peaks while these peaks are not significant in the mass spectra of its meta and para isomers. Deuterium –labeling analysis [14,15] have shown the exclusive involvement of methyl hydrogens in the formation of [M - OH]+ from the molecular ion of 2-nitrotoluene. Based on the MIKE spectral studies, Beynon and co-workers [16] have proposed that the most likely structure for the [M - OH]+ ion of 2-nitrotoluene is the 1,2-benzisoxazolium cation (Scheme 1.5.2).
McLuckey and Glish [17] compared and contrasted the tendency to lose OH$^-$ from the molecular cations and anions of 2-nitrotoluene, 2-nitrophenol and 2-nitroaniline. Suryanarayana and Axenrod [18] have reported that the [M - OH]$^+$ ion formed by a similar interaction from the molecular ion of 2,4,6 trinitrotoluene, plays an important role in the fragmentation path of this compound. Yinon [19] has carried out a CAD study of a series of 2,4,6 trinitro aromatic compounds and observed that the loss of OH$^-$ and NO are the dominant fragmentations.

Similar ortho interactions resulting in the expulsion of OH radical has been reported in the mass spectra of 1-nitro-2-methylnaphthalene [20], 7-nitrooxindole [21], 3-nitro-4-methyl pyrazole [22] and nitro methyl phenothiazines [23]. Bowie and co-workers [24] have explained the formation of [M-OH]$^+$ ion in the mass spectrum of N-2-nitrobenzylideneaniline due to interaction between the o-nitro and azomethene groups which was supported by deuterium labeling studies.
Johnstone and co-workers [25] have suggested a mechanism for the loss of OH radical in the EI mass spectral fragmentations of substituted N-o-nitrobenzylidiene anilines. (Scheme 1.5.3)

\[ \text{Scheme 1.5.3} \]

Holzmann and Rothkopf [26] have also reported the formation of \([M - \text{OH}]^+\) ion from the molecular ion of o-nitrobenzylidiene-anil of diaminodicyanoethane by a similar process. They have proposed a very interesting through-space interaction in the molecular ion resulting in the formation of \([M - \text{H}_2\text{O}]^+\) (Scheme 1.5.4)
Lord and Millard [27] have observed an interesting ortho interaction in the EI mass spectrum of 4-methyl nitroimidazole, where abundant ions are due to [M -OH]$^+$ and [M – H$_2$O]$^+$. Benoit and Holmes [28] have reported the loss of a water molecule from the molecular ion of 2-nitrophenyl hydrazine and 2,4-di nitro phenyl hydrazine. They have observed that D$_2$O is exclusively lost from $N, N'$-Tri deutero isomers on EI. This established the involvement of hydrogens in the side chain in the formation of [M – H$_2$O]$^+$ (Scheme.1.5.5). It is further observed that [(M – H$_2$O) –NO]$^+$ and [(M – H$_2$O) –N$_2$]$^+$ which are also noticed in the EI mass spectra of 2-nitrophenyl hydrazine and 2,4 - dinitro phenyl hydrazine are totally absent in their para isomers.
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Beynon and coworkers [13] and Harley-Mason and coworkers [29] have noticed the loss of CO from the molecular ion of 1-nitronaphthalene, which is not seen in the EI mass spectra of nitrobenzene and 2-nitro naphthalene. Moreover, 1-nitronaphthalene having substituents in the position 8- does not eliminate CO on EI. To explain these mechanisms, two mechanisms have been suggested [13, 29] for the formation of [M-CO]+ in the mass spectrum of 1-nitronaphthalene (Scheme 1.5.6) which involves the initial migration of the C8 hydrogen either to the oxygen (Path. A) or to the C9 carbon (Path. B). Path B is favored by Britain and co-workers [30] to explain the decompositions observed in the EI mass spectra of dinitro naphthalenes except in 1,3-dinitro naphthalene.

Scheme 1.5.6
Mayerson and coworkers [14] have reported the ejection of OH radical from the molecular ion of 2-nitro biphenyl on electron impact, (Scheme.1.5.7). They have suggested that the steric accessibility of the 2’-hydrogen allows the formation of \([M- OH]^+\) which is supported by the fact that 3- and 4-nitro biphenyls are devoid of this ion in their EI mass spectra. Another interesting proximity effect observed in fragmentation of 2-nitrobiphenyl on EI is the sequential loss of two CO molecules from the molecular ion. (Scheme.1.5.7)

![Scheme 1.5.7](image-url)
Thomas and Wilson [31] have also observed similar interaction of 2′-nitro group in dinitro biphenyls and trinitro biphenyls except in 2,2′- dinitro biphenyl where the displacement of one of the nitro group by a radical substitution mechanism results in the base peak of the EI mass spectrum (Scheme 1.5.8).

![Scheme 1.5.8](image)

The formation of the abundant [M-OH]$^+$ ion involving the migration of the hydrogen of the heterocyclic ring to the oxygen of the nitro group has been reported [32] in the mass spectra of 2-nitrophenyl imidazole and 2-nitrophenyl pyrazole. Robinson and coworkers [33] have observed that OH$^-$ loss from the molecular ion of 2-nitrodiphenyl methane involves the methylene hydrogen as well as the aromatic hydrogen at 2′-position. Jauregui and Lehmann [34] have reported an interannular hydrogen migration followed by cyclisation to phenoxazine-$N$-oxide with the expulsion of an OH radical in the EI mass spectral fragmentation process of 2,4-dinitrophenyl phenyl ether (Scheme 1.5.9).

![Scheme 1.5.9](image)
Bursey [35] has observed the formation of an ion corresponding to \([M - \text{CH}_3\text{OH}]^{+}\) directly from the molecular ion of dimethyl acetal of o-nitro benzaldehyde. It has been shown that the secondary fragmentations of the \([M - \text{CH}_3\text{OH}]^{+}\) are similar to the mass spectral decompositions of methyl-o-nitrosobenzoate on electron impact. A mechanism, analogous to the formation of methyl-o-nitrosobenzoate by photochemical [36] reaction of dimethyl acetal of o-nitro benzaldehyde is proposed to explain the formation of \([M - \text{CH}_3\text{OH}]^{+}\) (Scheme 1.5.10). The CAD studies by Schwarz [37] have supported the proposed structure for \([M - \text{CH}_3\text{OH}]^{+}\).

![Molecular Structures](image)

**Scheme 1.5.10**

Vijfhuizen and co-workers [38] have observed the expulsion of HNO directly from the molecular ion of 2-nitro benzaldoxime. Deuterium labeling studies have established that the hydrogen atom of the original hydroxyl function is involved in the HNO loss. Based on MIKE studies a mechanism has been proposed, (Scheme 1.5.11). Based on CAD analysis Schwarz [37] has suggested a ring structure ‘a’ for \([M - \text{HNO}]^{+}\).
Benoit and Holmes [10] have reported that the molecular ion of 2-nitrobenzyl alcohol loses a molecule of water while meta and para isomers do not. Deuterium labeling [10] studies have revealed the non-involvement of aromatic hydrogens and $^{18}$O- labeling studies [39] established the participation of the oxygen atom of the nitro group in the elimination of H$_2$O. Klair and co-workers [39] have observed the elimination of HCN from the molecular ion of 2-nitrobenzyl cyanide, along with the elimination of OH$^-$. They have also noticed that DCN is exclusively lost from its L-D$_2$ isomer, while [M-HCN]$^+$ is absent in the mass spectrum of $p$-nitro benzyl cyanide. Further, Schwarz [37] has established by CAD studies that the [M-H$_2$O]$^-$ from 2-nitrobenzyl alcohol and [M-HCN]$^+$ from 2-nitrobenzyl cyanide have the same structure that of 2,1-benzisoxazoline-3-one (Scheme 1.5.12).
However, detailed mechanism concerning these processes still remains inconclusive [9]. Tomer and co-workers [40] and Mamer and co-workers [41] have shown by high-resolution data that the ion at \( m/z \) 123 in the mass spectrum of 2-nitroanisole is formed mostly by the expulsion of CH\(_2\)O (80%) and the rest (20%) by the loss of NO\(^{-} \) from the molecular ion. The complete retention of the label in the \([M-CH_2O]^+\) fragment from 2-nitroanisole – \(^{18}\)O-CH\(_3\) established the elimination of an oxygen atom from the nitro group in the formation of \([M-CH_2O]^+\). The mechanism (Scheme 1.5.13) is consistent with this observation.

Scheme 1.5.13

Benoit and Homes [10] have observed that the mass spectrum of 2-nitro benzoic acid contains a peak corresponding to a fragment due to the loss of two CO molecules from \([M-NO_2]^+\). These ions are insignificant in the case of p-nitro benzoic acid. A mechanism involving the migration of OH from the –COOH function to the vacant (charge carrying) ortho position in the \([M-NO_2]^+\) ion followed by the ejection of CO, has been proposed (Scheme 1.5.14). Tomer and co-workers [40] have supported this mechanism by \(^{13}\)C-labelling.

Scheme 1.5.14
Aromatic nitro amines normally [42] eliminate the \( \text{NO}_2 \) radical from the molecular ion followed by the ejection of other substituents if present, or HCN by the rupture of the aromatic ring. However when another nitro group is present adjacent to nitramine function, \([\text{M-NO}_2]^+\) ion in these compounds is shown to eliminate two \( \text{NO} \) radicals [42] consecutively. This suggests that one of the oxygen atoms of the nitro group has become bonded to the remaining nitrogen atom of the nitramine function in \([\text{M-NO}_2]^+\) fragment due to their proximity (Scheme 1.5.15).

![Scheme 1.5.15]

Another classical proximity of the nitro group is illustrated in the migration of oxygen atom to the \( \alpha, \beta \) or \( \beta, \gamma \) - double bonds or to the atoms like N, S, C etc. Migration of an oxygen atom from the \textit{ortho} nitro group to the \(-\text{CH=N}-\) moiety followed by several rearrangements has been reported by Seibl and Vollmin [43] in the EI mass spectral fragmentations of 2,4,dinitro phenyl hydrazones of aromatic aldehydes. Here the rearranged molecular ion breaks up giving some of the most characteristic fragment ions due to the oxygen transfer in the mass spectra of these compounds (Scheme 1.5.16).

2,4-Dinitrophenyl hydrazones of aliphatic aldehydes and ketones [44 - 47] have also been observed to fragment by a similar \textit{ortho} interaction. Cable and co-workers [48] have reported an interesting \textit{ortho} interaction during the secondary fragmentation the ion at \( m/z \) 137
resulting in the formation of an intense fragment \( m/z \) 119 in the mass spectrum of 2,4-dinitrophenyl hydrazone of 2-methoxy benzaldehyde (Scheme 1.5.17).

Scheme 1.5.16

Scheme 1.5.17
Johnston and co-workers [25,49] have reported that $[\text{M-OH}]^+$, which is a prominent fragment in the EI mass spectrum of N-2-nitrobenzylidenaniline [24] is absent in the mass spectra of $N$-benzylidene 2-nitro anilines. They have observed that a fragment corresponding to the Aroyl cation dominated in the mass spectra of these compounds. They have proposed a mechanism involving the migration of oxygen from the nitro group to the carbon atom of the azomethine function in the molecular ion to explain the formation of the Aroyl cation (Scheme 1.5.18.)

![Scheme 1.5.18](image)

Scheme 1.5.18

Oxygen migration from the *ortho* nitro group to the olefinic double bond on electron impact has been thoroughly investigated. Seibl and Vollmin [43] have reported the formation of interesting ions in the mass spectrum of 2,4-dinitro stilbene as a result of oxygen migration. Deuterium and $^{13}$C-labelling studies by Middleton and co-workers [50] have established that the formation of the abundant $[\text{M-CHO}]^+$ at $m/z$ 120 in the EI mass spectrum of 2-nitrostyrene involves an epoxide intermediate and that the carbon atom of the ‘CHO lost from the carbon atoms of the side chain. (Scheme.1.5.19).
Ramana and Vairamani [51] have observed that the EI induced fragmentations of 2’-nitro-4-stilbazole involves the initial migration of an oxygen atom from the nitro group to the olefinic double bond followed by the decomposition the epoxide intermediate to give fragments at \( m/z \) 197, 135, 134, 120, 119, 107 and 106 (Scheme.1.5.20). These ions are absent in the 3, and 4’-nitro stilbazoles. However the formation of \([\text{M-NO}_2]^+\) by an intramolecular cyclisation mechanism [51] is very important in the EI mass spectral fragmentations of 2’-nitro-2-stilbazole. The same authors have further extended [52] their study to the fragmentations of styryl nitro thiophenes on electron impact, where a similar epoxide intermediate has been postulated to explain the formation of substituted benzyl ion, aroyl cation and ions at \( m/z \) 171. They have further established, with the aid of Hammet plots, that the formation of substituted benzyl ion is concerted in nature. Analogous oxygen transfer on electron impact has been suggested [53] to explain the formation of \( \text{Ar-CO}^+ \), \( \text{ArCH}_2^+ \), and \([\text{M-C}_3\text{H}_3\text{N}_2\text{O}]^+\) in the mass spectra of 3-methyl-4-nitro-5-styrlisoxazoles. Similar oxygen migration has been reported in the electron impact mass spectrum of benzal-2-nitro acetophenone [54].
Mintas and co-workers [55] have observed a very interesting through space interaction between the nitro and the methyl groups in the EI mass spectral fragmentations of 2-nitro-2’-methyl stilbene, resulting in the formation of ions at *m/z* 194, 193, 192, 191, 179 and 120 in addition to the expected ions due to oxygen migration to the olefinic double bond (Scheme 1.5.21). The formation of these ions directly from the molecular ion has been established by metastable ion analysis.
Scheme 1.5.21

Martens and co-workers [56] have reported the formation of intense ions at \( m/z \) 139 and 134 in the EI mass spectrum of 4-tolyl-2-nitro thiobenzoate. These ions are insignificant in its meta and para isomers. A mechanism, which involves the migration of an oxygen atom from the nitro group to the sulphur, has been proposed to explain the formation of these ions in the ortho isomer (Scheme 1.5.22).

Scheme 1.5.22
Mallen and Smith [57] have studied a similar migration of an oxygen atom from the nitro group to the sulphur to explain the formation of fragments such as \([(M-\text{SO})-\text{NO}_2]^+\) and \([(M-\text{NO}_2-\text{CO})-\text{SO}]^+\) in the EI mass spectra of 2, 2'-dinitro phenyl sulphide.

The \([M-\text{SO}_2]^+\) fragment in the mass spectrum of 2-nitrophenyl 4-tolyl sulphide is proposed to be formed [58] as a result of double oxygen transfer from the nitro group to the sulphur. The loss of a hydrogen radical from the \([M-\text{SO}_2]^+\) ion leading to a cyclic structure forms the base peak in the mass spectrum (Scheme 1.5.23).

But the selenium analogue [59] of this sulphide contains, peak in the EI mass spectrum corresponding to single oxygen migration only. This difference in behavior is attributed to the different redox potentials of the neutral particles \(\text{SO}_2\) and \(\text{SeO}_2\). Grutzmacher and Ramana [60] have referred to the formation of \([M-\text{SO}]^+\) in the mass spectrum of \(N, N\)-dimethyl-2-nitro thiobenzamide as a result of similar oxygen transfer from the nitro group to the sulphur of the thiocarbonyl function.

Schwarz and Bohlmann [61] and later Larsen and co-workers [42] have reported a similar type of oxygen migration to the nitrogen atom in \(N, N\)-dialkyl-2-nitrobenzamides on electron impact. The formation of the ion at \(m/z\) 134 directly from 2-nitrobenzoyl piperidine on electron impact involves an oxygen transfer from the nitro group to the nitrogen of the amide moiety. This has been confirmed by metastable ion studies. This ion is insignificant in the case of the para isomer (Scheme 1.5.24)
Ramana and Vairamani [62] have noticed the formation of a fragment at \( m/z \) 105 (C\(_7\)H\(_5\)O) directly from the molecular ion of \( N\)-benzyl-2-nitro aniline on electron impact. A mechanism, involving the migration of an oxygen atom from the nitro group to the benzylic carbon followed by a simple cleavage has been proposed to explain the formation of benzoyl cation (Scheme.1.5.25).

The other interesting ions observed as a result of proximity interactions between the nitro group and \(-\text{NH-CH}_2-\) moiety are \([\text{M - OH}]^+\), \([\text{M - H}_2\text{O}]^+\), \([(\text{M-OH}) - \text{O}]^+\), \([(\text{M-OH}) - \text{NO}]^+\) etc.

Benoit and Holmes [28] have explained the formation of the ion corresponding to the protonated cyclohexanone at \( m/z \) 99 [47], in the EI mass spectra of 2,4 – dinitrophenylhydrazone of cyclohexanone by invoking the migration of an oxygen atom from
the nitro group to the cyclohexanone ring through a cyclic intermediate (Scheme.1.5.26). It has been observed that the ion at $m/z$ 99 is shifted to $m/z$ 100 in the $N$-$D$ isomer confirming the proposed mechanism.

![Scheme.1.5.26](image.png)

Ramana and co-workers [63] have reported an interesting proximity effect during the secondary fragmentation of 2-nitro-trichloroactanilide on electron impact. The ion at $m/z$ 121 formed from the $[M-CCl_3]^+$ fragment has been explained due to the interaction of the nitro group with the –NHCO- moiety leading to the elimination of CO$_2$ (Scheme.1.5.27).

![Scheme.1.5.27](image.png)
Berbalk and coworkers [64] have explained the formation of an intense ion at \( m/z \) 134 directly from the molecular ion of 2-nitrobenzopheneone by invoking the migration of an oxygen atom from the nitro group to the carbon atom of the aromatic ring (Scheme.1.5.28).

\[
\text{Scheme.1.5.28}
\]

Konnecke and coworkers [65] have reported that azoles and azoloazines containing 2-nitrophenyl groups produce an ion at \( m/z \) 134 directly from the molecular ion. This can be explained by a mechanism in which oxygen from the nitro group is proposed to migrate to the imino carbon through a cyclic five membered transition state. (Scheme.1.5.29)

\[
\text{Scheme.1.5.29}
\]

Ramana and Viswanathan [66] have reported very interesting competitive oxygen migrations from the nitro group to the nitrogen and sulfur atoms during the EI mass spectral fragmentation of \textit{ortho} nitro aromatic thioamides. A mechanism, involving the initial oxygen
migration from the nitro group to the nitrogen of the amide function through a six membered cyclic transition state followed by an $\alpha$-fission has been formulated for the formation of the ion at $m/z$ 150. A similar fragmentation with a $\beta$-hydrogen migration is envisaged for the formation of the ion at $m/z$ 151. The direct formation of these fragment ions from the molecular ion is supported by MIKE spectrum. Another competing process is the transfer of an oxygen atom from the nitro group to the sulfur of the thioamides moiety through a six membered transition state followed by the loss of SO from the molecular ion. The proposed mechanism for this process involves the initial migration of an oxygen atom from the nitro group to the sulfur followed by the attack of the nitro function on the carbon with the concomitant expulsion of the SO from the molecular ion leading to a cyclic fragment ‘a’ (Scheme.1.5.30). The loss of SO was supported by the high-resolution data.

![Scheme 1.5.30](image-url)
Pappalarado and Grazia [67] have investigated the electron-impact induced oxygen transfers in 2-nitrophenyl-2-pyridyl sulphides. They have noticed the transfer of oxygen atoms from the nitro group to the imino carbon of the heterocyclic ring. Minor skeletal rearrangements triggered by single or double oxygen transfer to the sulfur atom, followed by the loss of SO or SO$_2$ were also observed, (Scheme.1.5.31). Similar ortho effects are observed in the EI mass spectrum of S-nitrophenyl substituted 2,5-dimercapto-1, 3,4-thiazoles [68] and 5,5’-dimercapto-2, 2’-bis(1,3,4-thiadiazolyl) S [69].

![Scheme.1.5.31](image)

Wilson and Bowie [70] have extensively studied the negative ion EI mass spectrum of 2-nitro phenyl phenyl ethers, thioethers and 2-nitro diphenylamines. They have reported the loss of H$_2$NO$_2$ by the proximity effect of nitro group. M. George and coworkers [71] have reported an unexpected ortho interaction of the nitro group during the EI mass spectral fragmentations of N-arylidene 2-nitrobenzenesulfenamides, where the molecular ions expelled SO$_2$ and N$_2$ both in concerted and stepwise processes (Scheme.1.5.32). Loss of a
hydrogen or the substituent from this fragment leads to a very abundant ion in all the compounds. Based on chemical evidences and linked-scan studies, a 1,2-phenylenetropiylium cation [71] structure has been postulated for the [M-SO$_2$-N$_2$-H/substituent]$^+$ ion.

Scheme.1.5.32

M. George and coworkers [72] have reported an oxygen transfer from the nitro group to the C = C group, followed by a simple cleavage, affords intense fragments corresponding to 2-nitroso thiophenol at $m/z$ 139 and 2-nitroso thiophenoxy cation at $m/z$ 138, (Scheme.1.5.33) during the EI mass spectral fragmentation of allyl 2-nitrophenyl sulfide.
Scheme.1.5.33

A double oxygen transfer from the nitro group to the sulfur [73] followed by the ejection of SO$_2$H from the molecular ion of allyl o-nitrophenyl sulfide leads to the formation of the quinolinium cation of $m/z$ 130 (Scheme.1.5.34). These processes are supported by the high-resolution data, CAD, linked-scan spectra and chemical evidences.

Scheme.1.5.34

An oxygen transfer from the nitro group to the olefinic double bond [73] followed by a simple cleavage gives an intense fragment corresponding to o-nitroso phenylthio cation at $m/z$ 138, (Scheme.1.5.35) during the mass spectral fragmentations of $\alpha$- (2-nitrophenyl thio) cinnamic acid.
Scheme 1.5.35

Single and double oxygen transfers from the nitro group to sulfur lead to concerted ejections of \([SO + CO_2]\) and \([SO_2 + CO_2]\) from the molecular ion of this compound (Scheme 1.5.36).
M. George and coworkers [74] have observed a double oxygen migration to sulfur from the ortho nitro group leading to the eliminations of SO$_2$ and SO$_2$H from the molecular ions (Scheme 1.5.37) and single oxygen transfer to the olefinic double bond in the side-chain giving rise to the most abundant ion at $m/z$ 138 in 2-nitrophenyl styryl sulfides on electron impact. (Scheme 1.5.38).

Scheme 1.5.37

Scheme 1.5.38

M. George and coworkers [75] have reported an unusual expulsion of SO from the molecular ions of substituted diphenyl dithiocarbonates under electron-impact conditions. An initial aryloxy migration to sulfur, followed by further rearrangement, is proposed for this
process. (Scheme.1.5.39). The diaryl thioketone radical-cation structure, assigned for the [M-SO] ion, was confirmed through the collision-activated dissociation and B/E linked-scan spectra.

Scheme.1.5.39

Ritter and coworkers have reported a novel denitration of nitro aromatic compounds by aryl nitrile radical cations [76]. The denitration reaction was applied to trinitrotoluene as a possible diagnostic reaction for the presence for nitro aromatic explosives.

1.6. Gas-Phase intramolecular Cyclisations in Mass spectrometry.

The investigations of gas-phase rearrangements leading to cyclisations and gas-phase analogies for similar reactions in solution-phase have been fascinating fields for researchers in organic mass spectrometry. A variety of ortho effects to leading to novel cyclic products have been reported in the EI mass spectrometric studies of aromatic nitro compounds [11,14 25, 26, 31, 37,48 54, 55, 58, 63, 69, 72-75]. 2,4 dinitro phenyl phenyl ether on electron impact [34] has shown an interannular hydrogen migration followed by cyclisation to
phenoxazine-N-oxide, (Scheme 1.5.9). Ramana and coworkers utilized the mass spectral studies of such cyclisations of radical cations for the discovery of new synthetic routes [77-81] to heterocyclic compounds. Ramana and coworkers have used EI mass spectrometer as a probe for the synthesis [79] of 2-substituted–4(3H)-quinazolines from 2-benzamidobenzamide and their substituted analogues (Scheme 1.6.1).

![Scheme 1.6.1](image)

Ramana and coworkers have synthesized 2-sustituted benzoxazoles from N- (2-hydroxyphenyl) benzamide and 2-sustituted benzimidazoles [78] from N- (2-aminophenyl) benzamide utilizing the EI mass spectral ortho interactions as a guide tool (Scheme 1.6.2).

![Scheme 1.6.2](image)
Ramana and coworkers have also reported the synthesis of 2-sustituted 4-H-3, 1-benzoxazin-4-ones [81] based on mass spectral ortho interactions. Orlando and co-workers reported the elimination of acetic acid [82] from protonated 4,5 diacetoxy phenanthrene and 2,2’-diacetoxy biphenyl on fast atom bombardment (Scheme 1.6.3).

Scheme 1.6.3

Kingston and coworkers reported the chemical ionization mass spectrometric study of Claisen rearrangement of protonated ally phenyl ether [83] in the gas-phase analogous to solution chemistry. Ramana and Sudha studied a Claisen rearrangement and cyclisation in phenyl propargyl ethers under electron impact conditions [84]. The same authors reported gas-phase cyclisations and thio-Claisen rearrangements in the molecular ions of phenyl allenyl sulfides and phenyl propargyl sulfides in EI mass spectrometry [85]. Ramana and co-workers studied the EI-induced 3,3-sigmatropic rearrangements and cyclisation in phenyl
allenyl methyl ethers [86]. Vairamani and coworkers have reported a gas-phase Claisen rearrangement of allyl phenyl ether, its sulfur and selenium analogues on electron impact [87]. Gas phase cyclisation reactions of 1 2-aryl amino phenyl) alkaniminyls have been studied by R. Leardini and co-workers [88], M.R.M. Domingues and co-workers [89, 90] have reported a novel step-wise expulsion of two OH radicals from *Meso*-Tetra phenyl porphyrins, having a nitro group in the -β-pyrrolic position upon protonation in FAB mass spectrometry (Scheme 1.6.4).

**Scheme 1.6.4**

Eberlin and co-workers found that various acylium ions in gas phase react readily with benzonitrile by cyclisation via a double nitrile addition to form aromatic 1,3,5-oxadiazinium ions in analogy with solution chemistry [91]. Cooks and coworkers reported that aryl nitrenium ions formed in a mass spectrometer ion source react with ethyl vinyl ether and 1,3-dioxolanes to illustrate, a gas phase “synthesis” of indoles and benzomorpholines via formation of new C-N bonds [92]. A detailed review of gas-phase analogies for solution reactions by Eberlin has shown that gas-phase synthetic chemistry is of current interest [93].
Vairamani and coworkers used EI and CI mass spectrometry for the differentiation of isomeric substituted ortho and para nitro diaryl ethers [94]. The EI mass spectra of the ortho isomers showed distinct fragment ions such as [M-OH]+, [M-OH-O]+, [M-OH-CO]+ and [M-OH-NO]+ consequent of the cyclisation of the M+ ion to afford a dibenzo-1, 4-oxazine structure (Scheme 1.6.5).

Scheme 1.6.5
1.7. References:


   (b) For a review: Brunelle A., Della-Negre, Le Beyee Y. ‘*Progress in the time of flight measurements’* *Analysis*, 1992, 41, 121-158. (22 references)
   (c). For a review; Wolnik H.’*Time of flight mass analysers’* *Mass spectrom Rev*. 1993, 12, 83-114 (59 references).

(b) R. Boyd J. Am. Chem. Soc. 1997, 8(7), 1191-1192


