



Mass Spectrometry

part of the

Instrumental Analysis in (Bio)Molecular Chemistry

Course

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1. Introduction

In mass spectrometry, one generates ions from a sample to be analyzed. These ions are then separated and quantitatively detected. Separation is achieved on the basis of different trajectories of moving ions with different mass/charge (m/z) ratios in electrical and/or magnetic fields.

Mass-spectrometry has evolved from the experiments and studies early in the 20th century that tried to explain the behaviour of charged particles in magnetic and electrostatic force fields. Well-known names from these early days are J. J. Thompson *investigation into the behaviour of ionic beams in electrical and magnetic fields* (1912), A. J. Dempster *directional focussing* (1918) and F. W. Aston *energy focussing* (1919). In this way a refinement of the technique was achieved that allowed important information concerning the natural abundance of isotopes to be collected.

The first analytical applications then followed in the early forties when the first reliable commercial mass spectrometers were produced. This was mainly for the quantitative determination of the several components in complex mixtures of crude oil.

In the beginning of the sixties the application of mass-spectrometry to the identification and structure elucidation of more complex organic compounds, including polymers and biomolecules, started. Since then the technique has developed to a powerful and versatile tool for this purpose, which provides information partly complementary to and overlapping with other techniques, such as NMR.

It is perhaps surprising that a technique that at first sight does not appear to give more information than the weight of a particle should be so important, since it is difficult to imagine a more prozaic property of a molecule than its molecular weight. The controlled fragmentation of the initial molecular ions yield interesting information that can contribute to structure elucidation. In addition the weights can now be determined with sufficient accuracy to allow elemental compositions to be derived.

These lecture notes were first drafted in Dutch by Peter van Galen in 1992, when the only mass spectrometer in the Faculty of Science was the Department's double sector instrument. They were translated into English in 2005, and gradually updated by Peter van Galen and Martin Feiters to include the descriptions of new instruments based on new principles and with new applications.

1.1. Basic Principles

Though the principles of a modern analytical mass-spectrometer are easily understood this is not necessarily true for the apparatus. A mass spectrometer especially a multi-sector instrument is one of the most complex electronic and mechanical devices one encounters as a chemist. Therefore this means high costs at purchase and maintenance besides a specialized training for the operator(s).

Measurement principles. In Figure 1.1 the essential parts of an analytical mass spectrometer are depicted. Its procedure is as follows:

1. A small amount of a compound, typically one micromole or less, is evaporated. The vapour is leaking into the ionization chamber where a pressure is maintained of about 10^{-7} mbar.

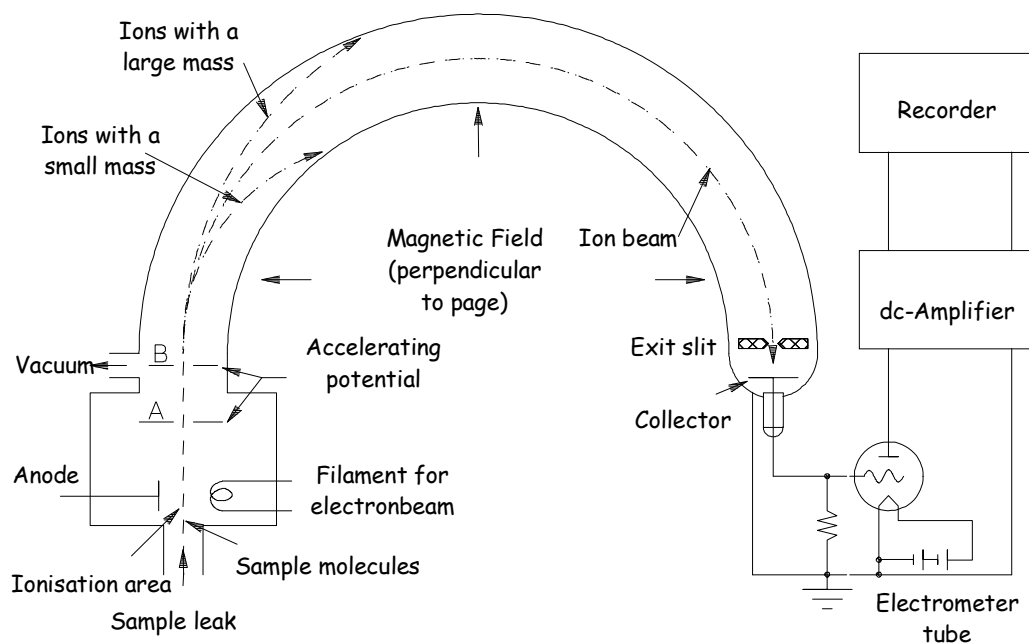


Figure 1.1. Schematic representation of a mass spectrometer

2. The vapour molecules are now ionized by an electron-beam. A heated cathode, the filament, produces this beam. Ionization is achieved by inductive effects rather than strict collision. By loss of valence electrons, mainly positive ions are produced.

3. The positive ions are forced out of the ionization chamber by a small positive charge (several Volts) applied to the repeller opposing the exit-slit (A). After the ions have left the ionization chamber, they 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 takes place in the analyzer, in this example a magnetic sector, at a pressure of about 10^{-8} mbar. A strong magnetic field is applied perpendicular to the motional direction of the ions. The fast moving ions then will follow a circular trajectory, due to the Lorentz 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.

The applicability of mass-spectrometry to the identification of compounds comes from the fact that after the interaction of electrons with a given molecule an excess of energy results in the formation of a wide range of positive ions. The resulting mass distribution is characteristic (a fingerprint) for that given molecule. Here there are certain parallels with IR and NMR. Mass-spectrograms in some ways are easier to interpret because information is presented in terms of masses of structure-components.

Sampling. As already indicated a compound normally is supplied to a mass-spectrometer as a vapour from a reservoir. In that reservoir, the prevailing pressure is about 10 to 20 times as high as in the ionization chamber. In this way, a regular flow of vapour-molecules from the reservoir into the mass spectrometer is achieved. For fluids that boil below about $150\text{ }^{\circ}\text{C}$ the necessary amount evaporates at room temperature. For less volatile compounds, if they are

thermally stable, the reservoir can be heated. If in this way sampling cannot be achieved one passes onto to direct insertion of the sample.

The quality of the sample, volatility and needed amount are about the same for mass-spectrometry and capillary gas chromatography. Therefore, the effluent of a GC often can be brought directly into the ionization chamber. Use is then made of the excellent separating power of a GC in combination with the power to identify of the mass-spectrometer. When packed GC is used, with a much higher supply of carrier-gas, it is necessary to separate the carrier gas prior to the introduction in the mass-spectrometer (jet-separator).

The Ion source. In Figure 1.2, the scheme of an ionization chamber, ion-source, typically electron impact, is presented. In this chamber in several ways, ions of the compound to be investigated can be produced. The most common way is to bombard vapour-molecules of the sample with electrons of about 70 eV. These electrons are generated by heating a metal wire (filament), commonly used are tungsten or rhenium. A voltage of about 70 Volts (from 5 to 100) accelerates these electrons towards the anode.

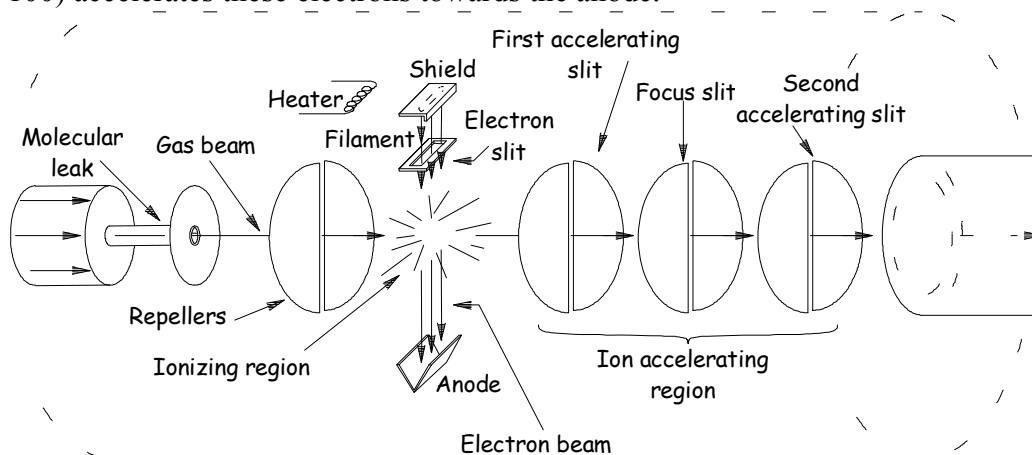


Figure 1.2. Schematic representation of an ion source.

During the bombardment, one or more electron can be removed from the neutral molecule thus producing positively charged molecular radical-ions. Only about one in 10^3 of the molecules present in the source are ionized. The ionization probability differs among substances, but it is found that the cross-section for most molecules is a maximum for electron energies from approximately 50 to 100 eV. Most existing compilations of electron impact spectra are based on spectra recorded with approximately 70 eV electrons, since sensitivity is here close to a maximum and fragmentation is unaffected by small changes in electron energy around this value. During this ionization, the radical-ions on average gain an excess energy enough to break one or more bonds and hence producing fragment-ions. In Figure 1.3 the possible fragmentation of a molecule ABCD is presented. It should be stated here that this is a simplified presentation and that in real life a multitude of possible ways to form fragments even via re-arrangement reactions exists. Fragmentation of a molecular radical cation to give a neutral molecule and a new fragment radical cation is also possible.

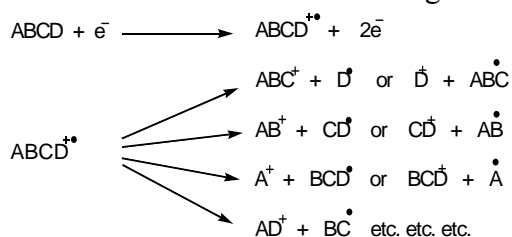


Figure 1.3. Possible fragmentation of a 'molecule' ABCD.

1.2. Gas Phase Ionization

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

Some general remarks on ionisation. When an electron with a kinetic energy, E_{kin} , of about 50 eV passes through a low-pressure gas mixture, as in the ion-source, an actual collision with a neutral molecule is not likely. The repelling force of the valence electrons can only be overcome when the E_{kin} is in the order of magnitude of 10^6 Volts. Therefore an electron will pass in 'short' distance of a molecule. A 50 eV electron travels with a speed of 4.2×10^8 cm/sec and passes a molecule, a few nanometers wide, in about 10^{-16} seconds. Due to the passing electric field the orbit of the valence electrons gets disturbed to such an extent that it may lose one valence electron and ionisation occurs, to give the radical cation of the molecule with molecular weight M:

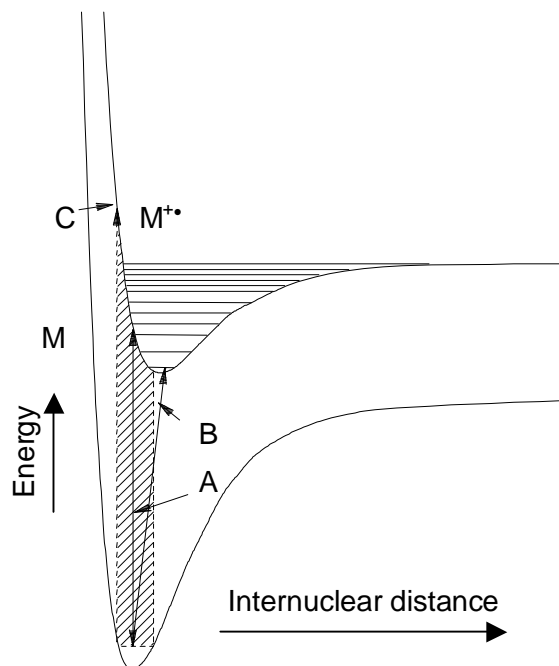
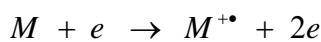


Figure 1.4: Ionisation process in a diatomic molecule.

The capture of an electron by neutral molecules is unlikely to happen. In practice it appears that in about 10^3 to 10^4 of the formed positive ions only one negative ion is formed.

The ionisation time of 10^{-16} second and the fastest vibrations in organic molecules, C-H stretch, indicate that the position of the nuclei may be considered unchanged during the process of ionisation.

The ionised level is reached through a so called Franck-Condon transition, the hatched area, arrow A, in Figure 1.4. As a result a molecular-ion is formed with several possible vibrational states. Some of them are at an high energy higher than the dissociation, arrow C in Figure 1.4, so that fragmentation occurs. A Franck-Condon transition can also allow molecular-ions in a higher electronic state to be formed. These will dissociate even easier, because of a decrease of the depth in the potential energy-curve and because the minima tend to drift to higher internuclear distances (Figure 1.5).

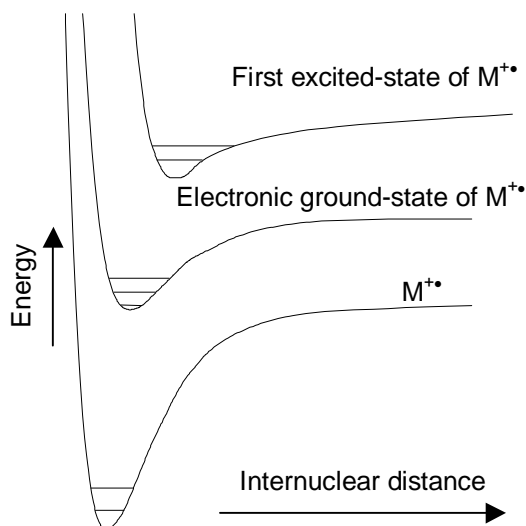


Figure 1.5: Potential energy curves in a diatomic molecule.

In poly-atomic molecules the change of the potential energy curves with changing internuclear distance is much more complicated. More oscillators are present and the curves will intersect at several points.

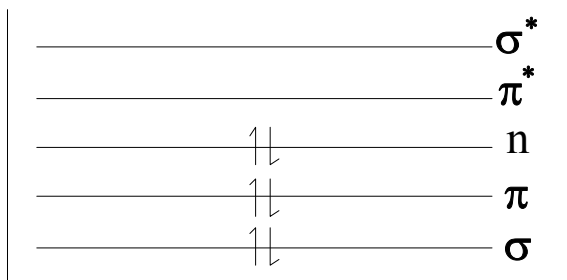


Figure 1.6: Electronic energy levels in a carbonyl group (schematic).

If we take acetone as an example and focus on the carbonyl we can distinguish three types of electrons namely n- (non-bonding), π - and σ -electrons. Their respective energy levels are depicted in Figure 1.6; this reveals that the probability of electron release during ionisation decreases in the order $n > \pi > \sigma$.

The ground-state of the molecular ion of acetone will be the one where a n-electron is eliminated. The first and second excited levels correspond to a missing π - or a σ -electron.

The majority of the molecular ions of acetone will lack a single electron and subsequent formation of fragment ions has to be explained from there. This is valid even if the primary ionisation was due to removal of a π or σ -electron. These ions cannot eliminate an excess of internal energy $E (= E_{el} + E_{vib} + E_{rot})$ through collisions with other molecules because of their formation in high vacuum. In the case of acetone and especially in larger molecules it is very probable that the energy curves of the first and second excited state of the molecular ion intersect. At this intersection the molecular ion can change its excited state, without energy loss. This radiation free transfer produces a highly excited molecular ion in the ground state which makes it even more prone to dissociation (Figure 1.7).

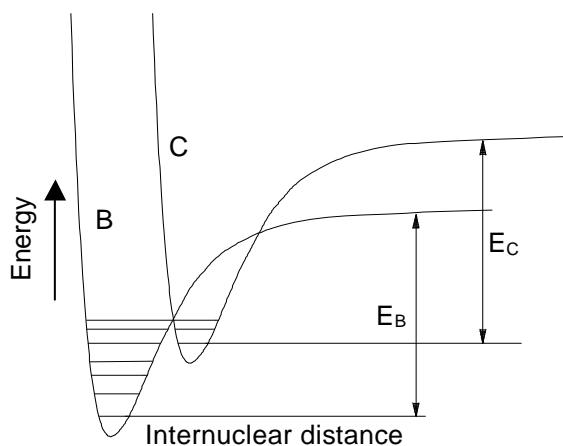


Figure 1.7: Intersecting potential energy curves.

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In practice mass spectra are recorded at 70 eV electron energy. This is because the amount of formed ions increases with increasing electron energy up to about 50 eV. Above 50 eV the amount of formed ions has reached a plateau and this is a condition to obtain reproducible mass spectra (Figure 1.8).

An energy of 70 eV corresponds to 1614 kcal/mole (1 eV = 23,06 kcal/mole). This is an enormous amount when compared to the average bond energy in organic molecules (Table 1.1). In addition the binding energy in ions will be much smaller. When you realise that electrons skim the molecule it is clear that not all 70 eV will be transferred.

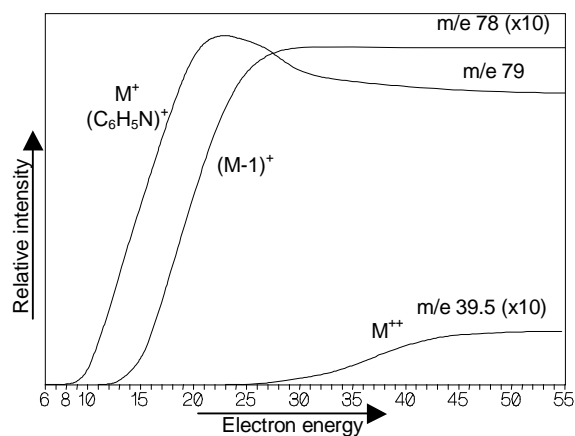


Figure 1.8: Relationship between ion-intensity and energy of the ionising electrons in the mass spectrum of pyridine.

Table 1.1: Energy content for some common bonds in organic chemistry (kcal/mol per bond)

| | Bond | | | | Bond | | |
|------|--------|--------|--------|------|--------|--------|--------|
| | Single | Double | Triple | | Single | Double | Triple |
| C-H | 98.7 | | | C-I | 51.0 | | |
| C-C | 82.6 | 145.8 | 199.6 | O-H | 110.6 | | |
| C-N | 72.8 | 147.0 | 212.6 | O-Si | 89.0 | | |
| C-O | 85.5 | 179.0a | | N-H | 93.4 | | |
| C-S | 65.0 | 128.0b | | N-N | 39.0 | 100.0 | |
| C-F | 116.0 | | | N-O | 53.0 | 145.0 | |
| C-Cl | 81.0 | | | S-H | 83.0 | | |
| C-Br | 68.0 | | | S-S | 54.0 | | |

a) ketones b) CS₂

Of course the ionisation energy is transferred from the fast electron to the molecule, otherwise no ionisation would occur. For organic molecules this is about 7 to 10 eV (Table 1.2, 1.3). Experimentally it is determined that besides the ionisation energy not more than 3 to 5 eV is transferred to the molecule. This amount is the internal energy E ($= E_{\text{el}} + E_{\text{vib}} + E_{\text{rot}}$) of the molecular ion ($\sim 70 - 115$ kcal/mole). In the ground state of the molecular ion of propane this means about 7 to 12 eV extra per bond (C₃H₈ 10 bonds). When accumulated in one bond this is enough to cause dissociation. It can be seen in Fig. 1.9 for benzoic acid as an example that the extent of fragmentation increases with the electron energy in electron impact ionisation, and has reached a stable level at 70 eV.

Table 1.2: Ionisation potential (eV) of some monofunctionalised aliphatic compounds CH₃CH₂CH₂X

| X = | Potential | X = | Potential |
|-------------------------------|-----------|--------------------|-----------|
| H | 11.07 | OH | 10.17 |
| ONO ₂ | 11.07 | CHO | 9.86 |
| Cl | 10.82 | CH=CH ₂ | 9.5 |
| CH ₃ | 10.63 | COCH ₃ | 9.34 |
| OAc | 10.54 | I | 9.26 |
| C ₂ H ₅ | 10.34 | NH ₂ | 8.78 |
| Br | 10.18 | | |

Table 1.3: As Table 1.2 for aromatic compounds C₆H₅X

| X = | a | b | c | X = | a | b | c |
|-------------------|------|-------|-------|----------------------------------|------|------|------|
| NO ₂ | 9.92 | 10.18 | 10.26 | CH ₃ | 8.82 | 9.18 | 8.9 |
| CN | 9.71 | 10.09 | 10.02 | I | 8.72 | 9.27 | 8.78 |
| CHO | 9.53 | 9.70 | 9.80 | OH | 8.50 | 9.16 | 8.75 |
| COCH ₃ | 9.27 | 9.57 | --- | SH | 8.33 | --- | --- |
| H | 9.24 | 9.56 | 9.40 | OCH ₃ | 8.22 | 8.83 | 8.54 |
| F | 9.20 | 9.73 | 9.50 | NH ₂ | 7.70 | 8.32 | 8.04 |
| Cl | 9.07 | 9.60 | 9.31 | NHCH ₃ | 7.35 | --- | 7.73 |
| Br | 8.93 | 9.52 | 9.25 | N(CH ₃) ₂ | 7.14 | 7.95 | 7.51 |

a = Photo-ionisation, b = Electron Impact, c = Photo-electron spectroscopy

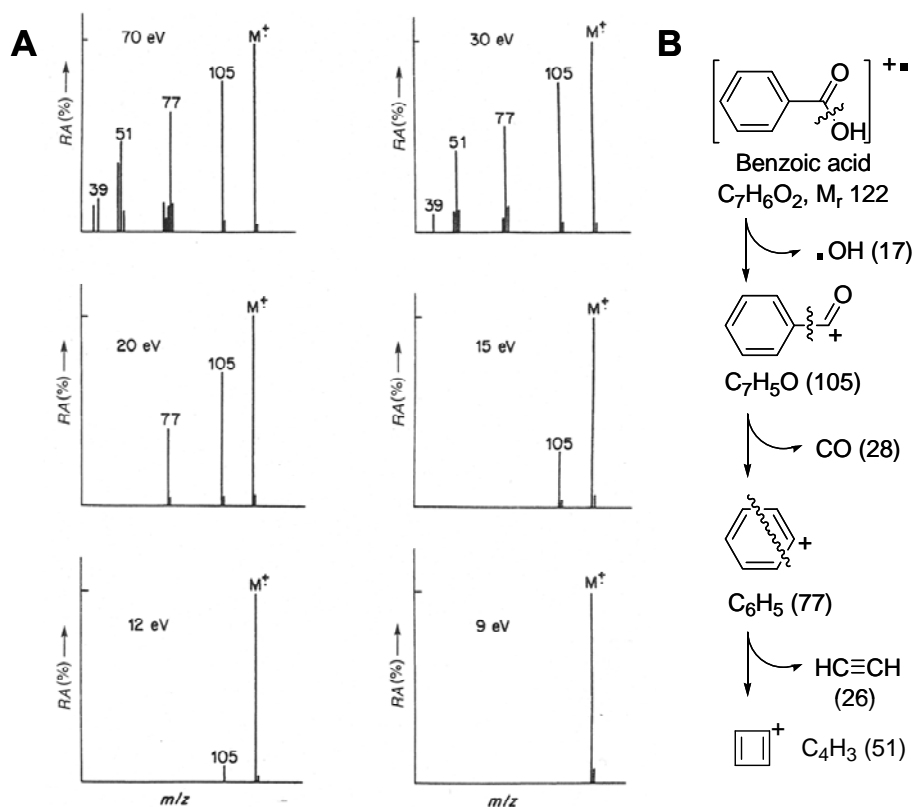


Figure 1.9. A) Relative intensities of the molecular ion and fragments of benzoic acid in electron impact ionization at various electron energies (Barker); B) fragmentation of benzoic acid.

Electron Ionization (EI). In the preceding text it is assumed that ionization takes place via EI (Electron Impact also Electron Ionization). EI is the oldest and best characterized of all the ionization methods. EI probably is the most common used ionization technique and still is of major importance. The principles were already pointed out in section 1.1 and Fig. 1.2. The ionization process can either produce a *molecular ion* M^+ which is a radical cation (an odd-electron or OE ion) with the same molecular weight and elemental composition as the starting analyte, or it can produce a *fragment ion* which corresponds to a smaller piece of the analyte molecule. After ionization, the remaining energy is about 2-7 eV per molecule, leading to fragmentation of the ion which gives information about the original structure; this will be discussed in more detail in the Local Charge and Fragmentation section. The low intensity or even the total absence of the molecular ion and the complexity of the resulting spectrum are against EI. The *ionization potential* is the electron energy that will produce a molecular ion. The *appearance potential* for a given fragment ion is the electron energy that will produce that fragment ion. Most mass spectrometers use electrons with an energy of 70 electron volts (eV) for EI. Decreasing the electron energy can reduce fragmentation, but it also reduces the number of ions formed.

Sample introduction

- heated batch inlet
- heated direct insertion probe
- gas chromatograph
- liquid chromatograph (particle-beam interface)

Benefits

- 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

- sample must be thermally volatile and stable
- the molecular ion may be weak or absent for many compounds.

Mass range

- *Low* Typically less than 1,000 Da.

Chemical Ionization (CI). *Chemical ionization* uses ion-molecule reactions to produce ions from the analyte. The set-up is identical to that for EI (Fig.1.10), with an additional possibility to admit a reagent gas (or 'reaction gas') at a higher pressure (1 torr) than the analyte. The chemical ionization process begins when a reagent gas such as methane, isobutane, or ammonia is ionized by electron impact. Non-ionized reaction gas molecules react with the radical cation and its reaction products to produce strong Brønsted acids in the gas phase, viz. CH_5^+ and C_2H_5^+ , corresponding to the conjugated acids of methane and ethene, in the case of methane (Fig. 1.11). These can protonate analyte molecules, resulting in the formation of the $(\text{M}+1)^+$ molecular ion, MH^+ (an even-electron or EE ion). Whether this analyte molecular ion can undergo further fragmentation depends on the energy transferred with the proton upon ionisation. Protonation by a stronger Brønsted acid gives more energy transfer and therefore more fragmentation. This is determined by the choice of reagent gas, with Brønsted acid strength decreasing in the order CH_5^+ (from methane) > $\text{C}_4\text{H}_{11}^+$ (from isobutane) > NH_4^+ (from ammonia). The EI and CI results for ephedrine with methane as reagent gas are given in Fig. 1.12.

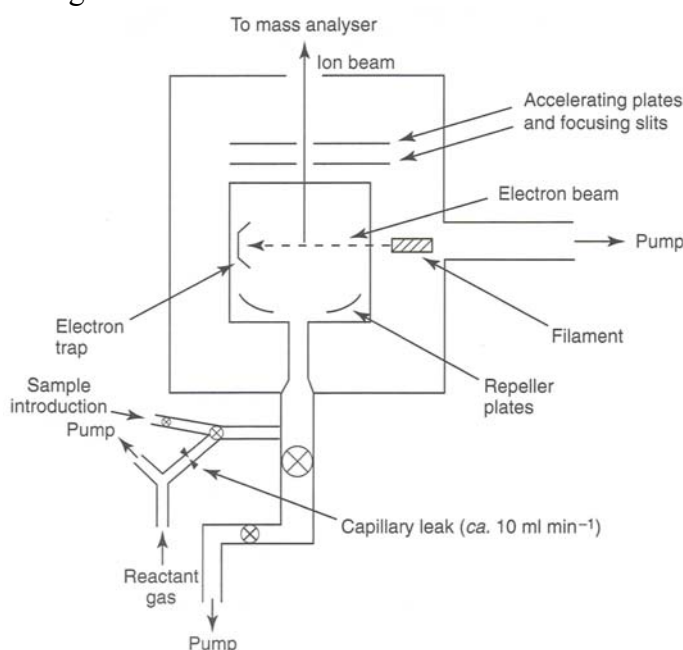


Figure 1.10 (Barker). Schematic representation of a chemical-ionisation ion source.

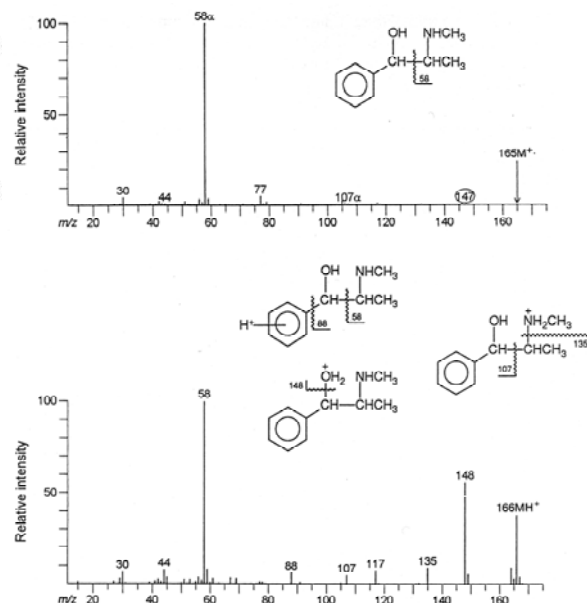
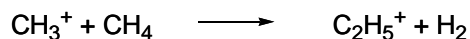
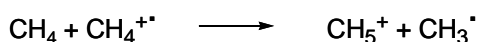
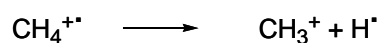
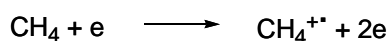
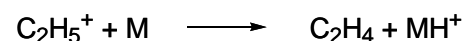
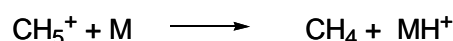


Figure 1.12. EI (top) and CI (methane, bottom) mass spectra of ephedrine.

a) Electron impact ionisation of reaction gas and subsequent formation of strong Brønsted acids



b) Reaction with proton-accepting analyte



c) Reaction with non proton-accepting analyte



Figure 1.11. Chemical ionisation with methane as a reaction gas.

Sample introduction

- heated batch inlet
- heated direct insertion probe
- gas chromatograph
- liquid chromatograph (particle-beam interface)

Benefits

- often gives molecular weight information through molecular-like ions such as $[\text{M}+\text{H}]^+$, even when EI would not produce a molecular ion.
- simple mass spectra, fragmentation reduced compared to EI

Limitations

- sample must be thermally volatile and stable
- less fragmentation than EI, fragment pattern not informative or reproducible enough for library search
- results depend on reagent gas type, reagent gas pressure or reaction time, and nature of sample.

Mass range

- *Low* Typically less than 1,000 Da.

Desorption Chemical Ionization (DCI). In this variation on chemical ionization, the analyte is placed on a filament that is rapidly heated in the CI plasma. The direct exposure to the CI reagent ions, combined with the rapid heating acts to reduce fragmentation. Some samples that cannot be thermally desorbed without decomposition can be characterized by the fragments produced by pyrolysis DCI.

Sample introduction

- sample deposited onto a filament wire
- filament rapidly heated inside the CI source.

Benefits

- reduced thermal decomposition
- rapid analysis
- relatively simple equipment

Limitations

- not particularly reproducible
- rapid heating requires fast scan speeds
- fails for large or labile compounds

Mass range

Low typically less than 1,500 D.

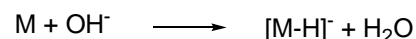
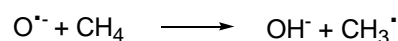
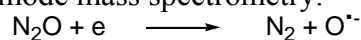
Negative-ion chemical ionization (NCI). Not all compounds will produce negative ions. However, many important compounds of environmental or biological interest can produce negative ions under the right conditions. For such compounds, negative-ion mass spectrometry is more efficient, sensitive and selective than positive-ion mass spectrometry. Negative ions can be produced by a number of processes. *Resonance electron capture* refers to the capture of an electron by a neutral molecule to produce a molecular anion. The electron energy is very low, and the specific energy required for electron capture depends on the molecular structure of the analyte.

Electron attachment is an endothermic process, so the resulting molecular anion will have excess energy. Some molecular anions can accommodate the excess energy. Others may lose the electron or fall apart to produce fragment anions.

A buffer gas (usually a common CI gas such as methane) is used to slow down the electrons in the electron beam until some of the electrons have just the right energy to be captured by the analyte molecules. The buffer gas can also help stabilize the energetic anions and reduce fragmentation. This is really a physical process and not a true "chemical ionization" process.

An example (Figure 1.13) is the formation of the 'base' OH^- in the gas phase with $\text{CH}_4/\text{N}_2\text{O}$ as the reagent gas. A $[\text{M}-1]^-$ peak is observed in negative mode mass spectrometry.

Figure 1.13. Negative ion chemical ionisation with $\text{CH}_4/\text{N}_2\text{O}$.



Sample introduction

- Same as for CI

Benefits

- efficient ionization, high sensitivity
- less fragmentation than positive-ion EI or CI
- greater selectivity for certain environmentally or biologically important compounds

Limitations

- not all volatile compounds produce negative ions
- poor reproducibility

Mass range

- *Low* Typically less than 1,000 D.

1.4. Isotopes, Satellite Peaks, Resolution

The possibility to measure weights of individual particles confronts us with the existence, for certain elements, of multiple isotopes, i.e. atoms with identical nuclear charge Z , so belonging to the same element, but different atomic weight, due to the presence of an identical number of protons combined with a different number of neutrons. Because the weights of protons and

neutrons are almost similar, the atomic weights are near-integer (non-accurate) multiples of the weight of the proton. These so-called nominal weights are also used to indicate the isotope as a pre-superscript to the atomic symbol, see Table 1.5. Accurate atomic weights in atomic mass unit (amu) are defined as the ratio to the weight of ^{12}C , which is arbitrarily set to 12.000.

Table 1.5. Natural abundance and exact weights of some isotopes:

| Element | Isotope | Natural abundance | Isotope weight | I |
|------------|------------------|-------------------|----------------|---------------|
| Hydrogen | ^1H | 99.985 % | 1.007 825 | $\frac{1}{2}$ |
| | ^2H (D) | 0.015 % | 2.014 102 | 1 |
| Carbon | ^{12}C | 98.9 % | 12.000 000 | 0 |
| | ^{13}C | 1.1 % | 13.003 354 | $\frac{1}{2}$ |
| Nitrogen | ^{14}N | 99.64 % | 14.003 074 | 1 |
| | ^{15}N | 0.36 % | 15.000 108 | $\frac{1}{2}$ |
| Oxygen | ^{16}O | 99.8 % | 15.994 915 | 0 |
| | ^{17}O | 0.04 % | 16.999 133 | $\frac{5}{2}$ |
| | ^{18}O | 0.2 % | 17.999 160 | 0 |
| Fluorine | ^{19}F | 100 % | 18.998 40 | $\frac{1}{2}$ |
| Silicon | ^{28}Si | 92.21 % | 27.976 93 | 0 |
| | ^{29}Si | 4.70 % | 28.976 49 | $\frac{1}{2}$ |
| | ^{30}Si | 3.09 % | 29.973 76 | 0 |
| Phosphorus | ^{31}P | 100 % | 30.973 76 | $\frac{1}{2}$ |
| Sulfur | ^{32}S | 95.0 % | 31.972 07 | 0 |
| | ^{33}S | 0.76 % | 32.971 46 | $\frac{3}{2}$ |
| | ^{34}S | 4.22 % | 33.967 86 | 0 |
| | ^{36}S | 0.014% | 35.967 09 | 0 |
| Chlorine | ^{35}Cl | 75.8 % | 34.968 855 | $\frac{3}{2}$ |
| | ^{37}Cl | 24.2 % | 36.965 896 | $\frac{3}{2}$ |
| Bromine | ^{79}Br | 50.5 % | 78.918 348 | $\frac{3}{2}$ |
| | ^{81}Br | 49.5 % | 80.916 344 | $\frac{3}{2}$ |
| Iodine | ^{127}I | 100 % | 126.900 4 | $\frac{5}{2}$ |

A consequence of the existence of isotopes is that molecular and fragment ions that contain certain elements in an isotope distribution corresponding to the expected natural abundance will display patterns of so-called satellite peaks. Interesting examples are some of the halogens, viz. Cl which occurs as the ^{35}Cl and ^{37}Cl isotopes in an approximate 3:1 ratio, while Br is found as ^{79}Br and ^{81}Br in an approximate 1:1 ratio (Table 1.5; Figure 1.14). These elements are typical examples of 'A+2' elements, i.e. elements characterized by the occurrence of another isotope which is 2 mass units heavier. Other examples of A+2 elements are O, S, and Si, although in the last case the intensity at A+1 is actually higher than that at A + 2. Table 1.6 also shows examples of 'A+1' elements such as C and N, and A elements (with no significant isotope distribution) such as H, F, P, and I. As the natural abundance of ^{13}C is 1.1 % of that of ^{12}C , any compound with mass M that contains x C atoms should have a (M+1) satellite peak with an intensity of about 1.1 x% of that of the M peak.

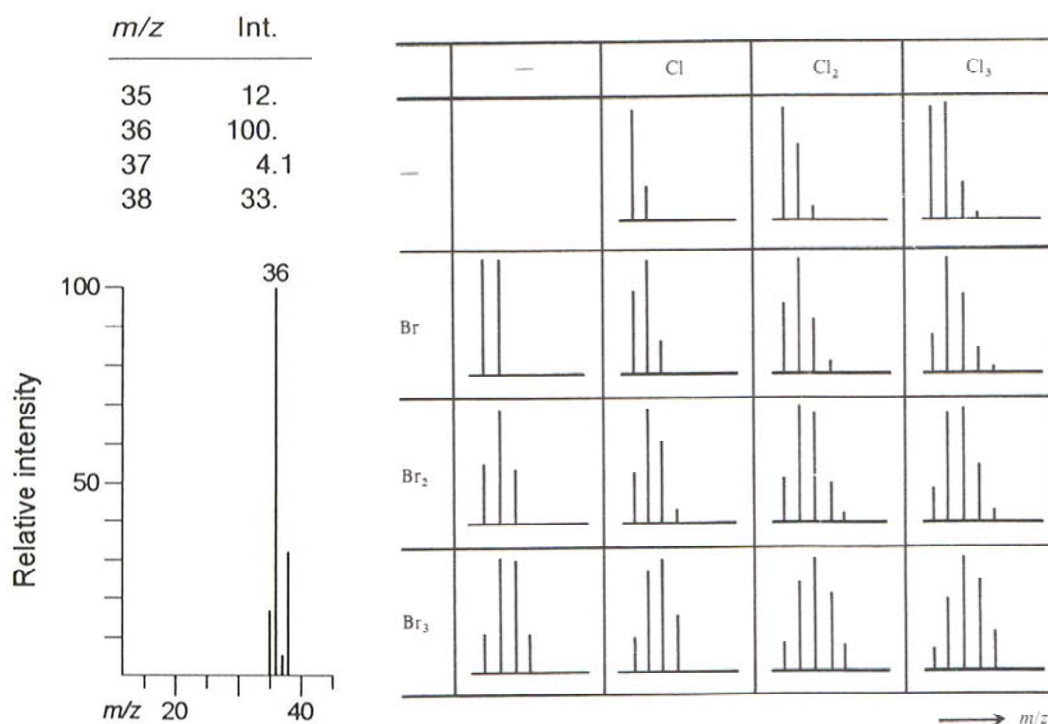


Figure 1.14. Satellite peak patterns in (left) HCl, (right) compounds with multiple Cl and/or Br (Williams).

Table 1.6. Natural abundance, distribution of isotopes, and satellite peak patterns, based on nominal (non-accurate) weights.

| | A | | A+1 | | A+2 | | Type of |
|---------|--------|-----|--------|-------|--------|------|---------|
| Element | Weight | % | Weight | % | Weight | % | element |
| H | 1 | 100 | 2 | 0.015 | - | - | A |
| C | 12 | 100 | 13 | 1.1 | - | - | A+1 |
| N | 14 | 100 | 15 | 0.37 | - | - | A+1 |
| O | 16 | 100 | 17 | 0.04 | 18 | 0.2 | A+2 |
| F | 19 | 100 | - | - | - | - | A |
| Si | 28 | 100 | 29 | 5.1 | 30 | 3.4 | A+2 |
| P | 31 | 100 | - | - | - | - | A |
| S | 32 | 100 | 33 | 0.79 | 34 | 4.4 | A+2 |
| Cl | 35 | 100 | - | - | 37 | 32.0 | A+2 |
| Br | 79 | 100 | - | - | 81 | 97.3 | A+2 |
| I | 127 | 100 | - | - | - | - | A |

The multiplicity and intensities of the satellite peak pattern can be calculated with $\binom{n}{k}$ of Newton's binomium: $(a + b)^n = a^n + n \cdot \frac{a^{n-1} \cdot b}{1!} + n(n-1) \cdot \frac{a^{n-2} \cdot b^2}{2!} + \dots$

For the example with 3 Br atoms, with relative abundance approx. $a = b = 1 : 1$, this gives

$$(a + b)^3 = a^3 + 3a^2b + 3ab^2 + b^3 = 1 + 3 + 3 + 1$$

i.e. 4 peaks with intensity ratio $1 : 3 : 3 : 1$. In general, for a combination of element x, in ratio $a : b$, combined with m atoms of element y, with ratio $c : d$, use the coefficients of

$$(a + b)^n (c + d)^m$$

The resolution of even the mass spectrometers with the simplest ion separation devices is enough to separate ions by nominal mass, but when we study the atomic weights in more detail, we realize that to have a resolution by which accurate masses would be determined to a few decimals would allow us to decide on the elemental composition of the molecular or fragment ion under study. For example, the exact mass of an ion with nominal $m/z = 29$ would be 29.03915, 29.01403, or 29.00273, depending on whether the elemental composition is respectively C_2H_5 , HCO , or N_2H .

Resolution. The resolution of a mass spectrometer can be defined by its ability to separate ions of adjacent mass number. Several definitions of resolution are used in mass spectrometry. It is useful to understand the distinctions between the different definitions in order to understand the characteristics of the different mass spectrometers.

Unit resolution means that you can separate each mass from the next integer mass. That is, one can tell the difference from masses 50 and 51 as from 1000 and 1001. This definition commonly is used for quadrupole and ion trap mass spectrometers, where the peaks usually are "flat-topped". In magnetic sector mass spectrometry resolution usually is defined as:

$$R = \frac{M}{\Delta M} \quad (15)$$

That is, the difference between two masses that can be separated divided by the mass number of the observed mass. In magnetic sector instruments, peaks usually are triangular or Gaussian (see Figure 1.15). Peaks in magnetic sector mass spectrometers usually are called separated to

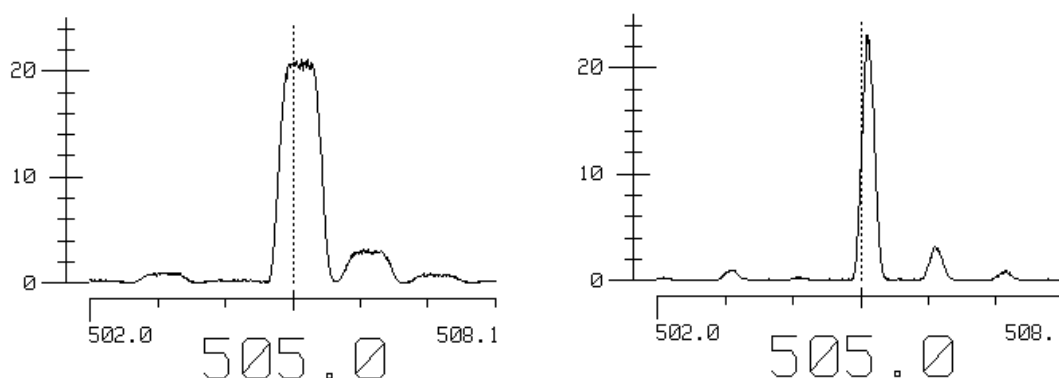


Figure 1.15: Flat-topped peaks e.g. quadrupole (left, ~unit resolution at 500) and triangular/ Gaussian peaks as from a magnetic sector mass spectrometer (right, ~1000 resolution)

a 10% valley when the overlap point is at 1/10 of the height of the higher of the two peaks. If only one peak is available, resolution is determined by the quotient of the observed mass divided by its width at the 5% level. The resolution is constant across the mass range.

Fourier Transform Ion Cyclotron Resonance (FTICR) mass spectrometers (not discussed in these lecture notes) use the same definition of resolution as magnetic sector mass spectrometers. However, the 50% valley definition (Figure 1.16) is often used because of the



Figure 1.16: Two peaks resolved to 10% valley (left) and 50% valley (right).

broadening of the peaks near the baseline due to apodisation and Lorentzian peak shape. In addition, resolution in FTICR is inversely proportional to mass, so it is important to know at what mass the given resolution was obtained. In time-of-flight mass spectrometry, the 50 % valley definition is used. Peak shapes in TOF are Gaussian.

Compare the unit resolution, as defined in quadrupole, and the resolution as defined in sector mass spectrometry. At 5000 resolution, in a magnetic sector instrument, one can distinguish between m/z 50.000 and m/z 50.010 or between m/z 1000.000 and m/z 1000.200. All separated to 10% valley. Unit resolution allows you to distinguish m/z 50 from m/z 51 or m/z 100 from m/z 101.

Some remarks on elemental composition calculations. The elemental composition of a molecular ion or fragment ion can be determined by accurately measuring its m/z value. Normally this is achieved by acquiring a mass spectrum at high resolution in order to measure the mass of a single species and not (partially) resolved peaks. Typically a resolution of 10000 (10% valley) is considered desirable for accurate mass spectrometry.

Elemental composition calculations are used to determine all the possible elemental compositions of a given mass with specified tolerance. The calculation is usually (not always) done for m/z values obtained from high-resolution mass spectra.

The total number of possible elemental compositions increases with mass and tolerance. Even for modest masses, the number can become very large. For m/z $146 \pm .5$ amu, containing C, H, N and O, this gives 33 possible compositions. For the same, with a tolerance of 0.005 amu, only containing C and H with possible N and O the number of possible structures is two. Doubling the mass gives 286 and 9 respectively, which shows the need of other constraints.

Limiting the number of possible elemental compositions can be achieved by:

- The accuracy of the measured mass.
- The set of elements and/or isotopes that are allowed in the elemental composition.
- The error limit for the calculated masses for the resulting elemental compositions.
- Upper and lower bounds on each allowed element and/or isotope.
- Methods of calculating that allow for odd-electron ions, even-electron ions or both.

Error Limits. Error limits can be used to limit the number of possible elemental compositions and are defined in terms of millimass units (*mmu*), unified atomic mass units (*u*), or parts-per-million (*ppm*). The parts-per-million error is defined as:

$$ppm = \frac{\text{measured mass} - \text{theoretical mass}}{\text{theoretical mass}} \cdot 10^6 \quad (16)$$

To overcome problems that arise when the theoretical mass becomes very small or large some additional limiting is necessary. The use of a low error bound and a high error bound makes it possible to define the limiting mass.

$$\text{Limiting mass} = \frac{mmu \cdot 10^{-3}}{ppm} \cdot 10^6 \quad (17)$$

Where *mmu* refers to the low or high error bound and *ppm* refers to the error limit as defined above. For example, the error limit is defined as 10 ppm; the low error bound as five *mmu* and the high error bound as 20 *mmu*. This gives the following error limits.

- 5 *mmu* for m/z < 500u

- 10 ppm for $500u \leq m/z < 2000u$
- 20 mmu for $m/z \geq 2000u$

When the error limit is expressed in *u* or *mmu* then no additional parameter is needed to define error limits in elemental composition calculations.

Isotope ratio measurements. As mentioned earlier (Table 1.5 and 1.6), most of the elements present in organic compounds have two or more stable isotopes. Information about isotope ratios can be used to limit the possible elemental compositions that correspond to a given measured mass. For example, a composition such as C_6H_5Cl is not reasonable for an m/z 112 ion unless there are chlorine isotope peaks at m/z 112 and m/z 114 with a relative ratio of about 3:1. The elemental composition calculation can be simulated with theoretical isotope ratio calculations. Such an approach is very useful for the interpretation of the mass spectra of inorganic or organometallic compounds. Many of these compounds have distinctive isotope patterns that allow the chemist to determine how many metal atoms (for example) are present in a particular ionic species.

1.5. Examples

Methyl stearate. Suppose that you measure the mass for a molecular ion and find it to be 298.285189 u, and that you expect to find carbon, hydrogen, nitrogen, and oxygen to be possible elements for the composition. If the limits for the calculation are set as displayed below, only one composition is possible for that measured mass: $C_{19}H_{38}O_2$.

```
Element Limits:  C 5/50   H 10/100   N 0/2   O 0/4
Tolerance:      10.00 PPM
Low error bound (mmu):  5.0 (for masses < 500)
High error bound (mmu): 20.0 (for masses < 2000)
Even or odd electron ion or both: BOTH
Minimum unsaturation: -0.5
Maximum unsaturation: 10.0
```

| Meas. mass u | Abund. | Diff. ppm | Unsat. | Compositions |
|-----------------|--------|--------------|--------|---------------|
| 298.28519 | 0.00 | -6.75 | 1.0 | C19 H38 N0 O2 |

Chlorpyrifos. If there are many possible heteroatoms, the number of possible elemental compositions may be very large and it may be difficult to determine which is correct without additional information. An example is the insecticide *chlorpyrifos* which has the composition $C_9H_{11}Cl_3NO_3PS$. If we assume a large range for the elemental limits, then the number of compositions is very large:

```
Element Limits:
C 5/20   H 5/42   N 0/5   O 0/10   Cl 1/4   P 0/5   S 0/5
Tolerance:  5.00 PPM
Low error bound (mmu):  5.0 (for masses < 1000)
High error bound (mmu): 20.0 (for masses < 4000)
Even or odd electron ion or both: ODD
Minimum unsaturation: -0.5
Maximum unsaturation: 10.0
Rel. abundance cutoff (percent):  0.000
```

| Meas. mass u | Abund. | Diff. ppm | Unsat. | Compositions |
|-----------------|--------|--------------|--------|---|
| 348.924988 | 0.00 | -10.58 | 5.0 | C ₅ H ₅ N ₅ O ₇ Cl ₂ P ₀ S ₁ |

| | | | | | | | | |
|--------|-----|-----------------|-----------------|----------------|----------------|-----------------|----------------|----------------|
| -5.07 | 5.0 | C ₅ | H ₆ | N ₃ | O ₉ | Cl ₁ | P ₂ | S ₀ |
| 13.03 | 5.0 | C ₅ | H ₆ | N ₅ | O ₅ | Cl ₂ | P ₁ | S ₁ |
| -14.17 | 5.0 | C ₅ | H ₇ | N ₅ | O ₅ | Cl ₂ | P ₂ | S ₀ |
| 9.36 | 5.0 | C ₅ | H ₈ | N ₅ | O ₃ | Cl ₂ | P ₃ | S ₀ |
| -12.59 | 4.0 | C ₅ | H ₉ | N ₅ | O ₃ | Cl ₁ | P ₁ | S ₃ |
| 11.02 | 4.0 | C ₅ | H ₁₀ | N ₅ | O ₁ | Cl ₁ | P ₂ | S ₃ |
| -10.85 | 0.0 | C ₅ | H ₁₁ | N ₁ | O ₈ | Cl ₃ | P ₁ | S ₀ |
| 7.26 | 0.0 | C ₅ | H ₁₁ | N ₃ | O ₄ | Cl ₄ | P ₀ | S ₁ |
| 12.77 | 0.0 | C ₅ | H ₁₂ | N ₁ | O ₆ | Cl ₃ | P ₂ | S ₀ |
| 3.67 | 0.0 | C ₅ | H ₁₃ | N ₃ | O ₂ | Cl ₄ | P ₂ | S ₀ |
| -5.60 | 5.0 | C ₆ | H ₆ | N ₃ | O ₈ | Cl ₂ | P ₁ | S ₀ |
| 12.51 | 5.0 | C ₆ | H ₆ | N ₅ | O ₄ | Cl ₃ | P ₀ | S ₁ |
| -4.02 | 4.0 | C ₆ | H ₈ | N ₃ | O ₆ | Cl ₁ | P ₀ | S ₃ |
| 8.92 | 5.0 | C ₆ | H ₈ | N ₅ | O ₂ | Cl ₃ | P ₂ | S ₀ |
| -13.12 | 4.0 | C ₆ | H ₉ | N ₅ | O ₂ | Cl ₂ | P ₀ | S ₃ |
| -7.70 | 4.0 | C ₆ | H ₁₀ | N ₃ | O ₄ | Cl ₁ | P ₂ | S ₂ |
| 10.67 | 4.0 | C ₆ | H ₁₀ | N ₅ | O ₀ | Cl ₂ | P ₁ | S ₃ |
| -11.37 | 0.0 | C ₆ | H ₁₁ | N ₁ | O ₇ | Cl ₄ | P ₀ | S ₀ |
| 12.24 | 0.0 | C ₆ | H ₁₂ | N ₁ | O ₅ | Cl ₄ | P ₁ | S ₀ |
| -11.28 | 4.0 | C ₆ | H ₁₂ | N ₃ | O ₂ | Cl ₁ | P ₄ | S ₁ |
| 12.33 | 4.0 | C ₆ | H ₁₃ | N ₃ | O ₀ | Cl ₁ | P ₅ | S ₁ |
| -6.12 | 5.0 | C ₇ | H ₆ | N ₃ | O ₇ | Cl ₃ | P ₀ | S ₀ |
| 8.40 | 5.0 | C ₇ | H ₈ | N ₅ | O ₁ | Cl ₄ | P ₁ | S ₀ |
| 0.87 | 4.0 | C ₇ | H ₉ | N ₁ | O ₇ | Cl ₁ | P ₁ | S ₂ |
| -8.13 | 4.0 | C ₇ | H ₁₀ | N ₃ | O ₃ | Cl ₂ | P ₁ | S ₂ |
| -2.71 | 4.0 | C ₇ | H ₁₁ | N ₁ | O ₅ | Cl ₁ | P ₃ | S ₁ |
| -6.47 | 3.0 | C ₇ | H ₁₂ | N ₃ | O ₁ | Cl ₁ | P ₀ | S ₅ |
| -11.81 | 4.0 | C ₇ | H ₁₂ | N ₃ | O ₁ | Cl ₂ | P ₃ | S ₁ |
| -6.30 | 4.0 | C ₇ | H ₁₃ | N ₁ | O ₃ | Cl ₁ | P ₅ | S ₀ |
| -2.89 | 9.0 | C ₈ | H ₅ | N ₅ | O ₃ | Cl ₁ | P ₁ | S ₂ |
| -6.56 | 9.0 | C ₈ | H ₇ | N ₅ | O ₁ | Cl ₁ | P ₃ | S ₁ |
| 0.35 | 4.0 | C ₈ | H ₉ | N ₁ | O ₆ | Cl ₂ | P ₀ | S ₂ |
| -8.66 | 4.0 | C ₈ | H ₁₀ | N ₃ | O ₂ | Cl ₃ | P ₀ | S ₂ |
| -3.24 | 4.0 | C ₈ | H ₁₁ | N ₁ | O ₄ | Cl ₂ | P ₂ | S ₁ |
| -12.33 | 4.0 | C ₈ | H ₁₂ | N ₃ | O ₀ | Cl ₃ | P ₂ | S ₁ |
| -1.57 | 3.0 | C ₈ | H ₁₃ | N ₁ | O ₂ | Cl ₁ | P ₁ | S ₄ |
| -6.91 | 4.0 | C ₈ | H ₁₃ | N ₁ | O ₂ | Cl ₂ | P ₄ | S ₀ |
| -5.25 | 3.0 | C ₈ | H ₁₅ | N ₁ | O ₀ | Cl ₁ | P ₃ | S ₃ |
| -3.41 | 9.0 | C ₉ | H ₅ | N ₅ | O ₂ | Cl ₂ | P ₀ | S ₂ |
| 2.01 | 9.0 | C ₉ | H ₆ | N ₃ | O ₄ | Cl ₁ | P ₂ | S ₁ |
| -7.08 | 9.0 | C ₉ | H ₇ | N ₅ | O ₀ | Cl ₂ | P ₂ | S ₁ |
| -1.57 | 9.0 | C ₉ | H ₈ | N ₃ | O ₂ | Cl ₁ | P ₄ | S ₀ |
| -3.67 | 4.0 | C ₉ | H ₁₁ | N ₁ | O ₃ | Cl ₃ | P ₁ | S ₁ |
| -2.10 | 3.0 | C ₉ | H ₁₃ | N ₁ | O ₁ | Cl ₂ | P ₀ | S ₄ |
| -7.43 | 4.0 | C ₉ | H ₁₃ | N ₁ | O ₁ | Cl ₃ | P ₃ | S ₀ |
| 10.67 | 9.0 | C ₁₀ | H ₅ | N ₁ | O ₇ | Cl ₁ | P ₁ | S ₁ |
| 1.57 | 9.0 | C ₁₀ | H ₆ | N ₃ | O ₃ | Cl ₂ | P ₁ | S ₁ |
| 6.91 | 9.0 | C ₁₀ | H ₇ | N ₁ | O ₅ | Cl ₁ | P ₃ | S ₀ |
| 3.15 | 8.0 | C ₁₀ | H ₈ | N ₃ | O ₁ | Cl ₁ | P ₀ | S ₄ |
| -2.19 | 9.0 | C ₁₀ | H ₈ | N ₃ | O ₁ | Cl ₂ | P ₃ | S ₀ |
| -4.29 | 4.0 | C ₁₀ | H ₁₁ | N ₁ | O ₂ | Cl ₄ | P ₀ | S ₁ |
| -7.87 | 4.0 | C ₁₀ | H ₁₃ | N ₁ | O ₀ | Cl ₄ | P ₂ | S ₀ |
| 10.06 | 9.0 | C ₁₁ | H ₅ | N ₁ | O ₆ | Cl ₂ | P ₀ | S ₁ |
| 1.05 | 9.0 | C ₁₁ | H ₆ | N ₃ | O ₂ | Cl ₃ | P ₀ | S ₁ |
| 6.47 | 9.0 | C ₁₁ | H ₇ | N ₁ | O ₄ | Cl ₂ | P ₂ | S ₀ |
| -2.62 | 9.0 | C ₁₁ | H ₈ | N ₃ | O ₀ | Cl ₃ | P ₂ | S ₀ |
| 8.05 | 8.0 | C ₁₁ | H ₉ | N ₁ | O ₂ | Cl ₁ | P ₁ | S ₃ |
| 4.46 | 8.0 | C ₁₁ | H ₁₁ | N ₁ | O ₀ | Cl ₁ | P ₃ | S ₂ |
| 5.95 | 9.0 | C ₁₂ | H ₇ | N ₁ | O ₃ | Cl ₃ | P ₁ | S ₀ |
| 7.70 | 8.0 | C ₁₂ | H ₉ | N ₁ | O ₁ | Cl ₂ | P ₀ | S ₃ |
| 5.42 | 9.0 | C ₁₃ | H ₇ | N ₁ | O ₂ | Cl ₄ | P ₀ | S ₀ |

---CORRECT--->

If we take a look at the measured isotope ratios, we can put some more reasonable limits on the elements to be included. Suppose that a low resolution mass spectrum shows the following (ignoring any interferences or overlapping losses that might be found in a real mass spectrum):

| m/z | Rel. abundance % |
|------|------------------|
| ---- | ----- |
| 349 | 98.5 |
| 350 | 11.3 |
| 351 | 100 |
| 352 | 11.5 |
| 353 | 35.7 |
| 354 | 4.1 |
| 355 | 4.9 |
| 356 | 0.4 |

From this, we might conclude that there are between 8 and 11 carbons (because the ^{13}C peak should be 1.1% times the number of carbons) and three chlorines (because of the 349/351/353 isotope peaks). The maximum number of hydrogens is estimated as twice the number of carbons plus 2. These assumptions drastically reduce the number of possible compositions.

```

Element Limits:
C 8/11   H 5/24   N 0/5   O 0/10   Cl 3/3   P 0/5   S 0/5
Tolerance: 5.00 PPM
Low error bound (mmu): 5.0 (for masses < 1000)
High error bound (mmu): 20.0 (for masses < 4000)
Even or odd electron ion or both: ODD
Minimum unsaturation: -0.5
Maximum unsaturation: 10.0
Rel. abundance cutoff (percent): 0.000
Meas. mass      Abund.  Diff.  Unsat.  Compositions
   u                ppm
348.924988      0.00   -8.66    4.0    C8 H10 N3 O2 Cl3 P0 S2
                  -12.33    4.0    C8 H12 N3 O0 Cl3 P2 S1
                  -3.67    4.0    C9 H11 N1 O3 Cl3 P1 S1 <--
                  -7.43    4.0    C9 H13 N1 O1 Cl3 P3 S0
                   1.05    9.0    C11 H6 N3 O2 Cl3 P0 S1
                  -2.62    9.0    C11 H8 N3 O0 Cl3 P2 S0

```

It is still not easy to tell the correct composition. If we would have better mass accuracy, say, a 5 ppm error tolerance, then there are three compositions that are possible:

```

Meas. mass      Abund.  Diff.  Unsat.  Compositions
   u                ppm
348.924988      0.00   -3.67    4.0    C9 H11 N1 O3 Cl3 P1 S1 <--
                   1.05    9.0    C11 H6 N3 O2 Cl3 P0 S1
                  -2.62    9.0    C11 H8 N3 O0 Cl3 P2 S0

```

The final decision about the correct composition will depend on whether we know something about the unsaturation (4 or 9 rings and/or sites of unsaturation) and whether we know anything about the number of constituting elements.

Here classical elemental analysis may be helpful. With this technique, the amount of carbon, hydrogen, nitrogen and/or sulfur is determined in a sample. Through this, the purity and the elemental ratios are determined. Even it is possible, through calculus, to predict the composition of the remainder if the total of percentages does not add up to 100. The result should help in the selection of the right composition.

2. Charge Location and Fragmentation

2.1. Identification of the Molecular Ion. As we have seen in section 1.4, it is expected that a chemically pure compound contains a mixture of isotopes, based on the natural abundance. The molecular ion assigned on the basis of the most abundant isotope(s). The conditions for the assignment of M^+ are the following:

a) The peak assigned as M^+ should be the highest m/z in the spectrum, apart from weak satellite peaks that result from other isotopes. Problems can arise with unstable M^+ or chemical impurities.

b) With Electron Impact (EI) Ionization, M^+ should be a radical (odd-electron, OE) cation, as it is ionized by removing 1 e. This means that when the formula to determine the number of *double bond equivalents* (DBE) also known as the *degree of unsaturation*,

$$\text{DBE} = x - \frac{1}{2}y + \frac{1}{2}z + 1$$

is applied to the formula $C_xH_yN_zO_n$ (where C is any 4-valent element, H any monovalent, N any 3-valent, and O any divalent element), the number of DBE found should be an integer.

An alternative expression for the DBE is

$$D = 1 + \left[\frac{1}{2} \cdot \sum_i^{i_{\max}} N_i \cdot (V_i - 2) \right] \quad (18)$$

where D is the degree of unsaturation, i_{\max} is the total number of different elements in the composition, N_i the number of atoms of element i and V_i the valence of atom i .

Each ring and each double bond counts as one degree of unsaturation, each triple bond as two, and each phenyl ring as four (ring + 3 double bonds in the Kekule structure). Due to the division by two, the result can be either an exact integer or an integer with a remainder of a half. For the odd-electron radical cation M^+ generated with EI, an integer value should be obtained; a remainder of 0.5 indicates an even-electron ion such as the $(M+H)^+$ ion obtained with CI. The minimum value for D in organic chemistry is -0.5 which corresponds with a protonated saturated compound like H_3O^+ .

c) The value of M^+ is even unless there is an odd number of N. The so-called nitrogen rule says that an organic compound with an even mass has an even number of nitrogens. If the compound has an odd mass then it contains an odd number of nitrogens. This can be explained by the fact that every element with an odd mass has an odd valence and an element with an even mass has an even valence. Nitrogen is the exception having an odd valence and an even mass. Therefore, when an OE molecular radical cation with an odd mass is found in EI it should contain an odd number of nitrogens. For the EE $(M+H)^+$ molecular ion obtained with CI an even mass for a protonated molecular ion corresponds to an odd number of nitrogens.

d) If M^+ is correctly assigned, the other peaks at high m/z can be logically explained by loss of neutral parts or molecules. Typical losses that can be easily explained are that of CH_3 (M-15), H_2O (M-18), CH_3O (M-31), $CH_3C=O$ (M-43) etc. The occurrence of M-2 till M-14, M-21 till M-25, M-33, M-37, and M-38 would not be compatible with peak M as a molecular ion. An overview of general fragments from molecular ions is given in Table 2.1.

Table 2.1. General fragments from molecular-ions

| m/z | Fragment | Found with, possible chemical class. |
|--------------|--|---|
| M-1 | H | |
| M-2 | H ₂ | - |
| M-14 | - | - |
| M-15 | CH ₃ | - |
| M-16 | O, NH ₂ | Ar-NO ₂ , sulfoxides / ArSO ₂ NH ₂ , -CONH ₂ |
| M-17 | OH / NH ₃ | Alcohols / Amines |
| M-18 | H ₂ O | Alcohols, aldehydes, ketones etc. |
| M-19 / 26 | F / HF | Fluorine derivatives |
| M-26 | C ₂ H ₂ | Aromatic hydrocarbons |
| M-27 | HCN | Aromatic nitriles, nitrogen heterocyclics |
| M-28 | CO / C ₂ H ₄ | Quinones / Aromatic ethylethers, ethylesters, n-propylketones |
| M-29 | CHO / C ₂ H ₅ | - / ethylketones, Ar- <i>n</i> -C ₃ H ₇ |
| M-30 | C ₂ H ₆ /CH ₂ O / NO | - / aromatic methylethers / Aromatic NO ₂ |
| M-31 | OCH ₃ | Methyl esters |
| M-32 | CH ₃ OH / S | Methyl-esters / - |
| M-33 | H ₂ O + CH ₃ / | - / thiols |
| M-34 | H ₂ S | Thiols |
| M-41 | C ₃ H ₅ | Propyl-esters |
| M-42 | CH ₂ CO / C ₃ H ₆ | Methyl-ketones, Ar-Acetates, ArNHCOCH ₃ /n- or iso-butylketones, Ar-propyl ethers, Ar- <i>n</i> -C ₄ H ₉ |
| M-43 | C ₃ H ₇ / CH ₃ CO | Propyl-ketones, Ar- <i>n</i> -C ₄ H ₉ / methyl-ketones |
| M-44 | CO ₂ / C ₃ H ₈ | Esters (rearrangement) / anhydrides |
| M-45 | CO ₂ H / OC ₂ H ₅ | Carbonic acids / ethyl esters |
| M-46 | C ₂ H ₅ OH / NO ₂ | Ethyl esters / Ar-NO ₂ |
| M-48 | SO | Aromatic sulfoxides |
| M-55 | C ₄ H ₇ | Butyl esters |
| M-56 | C ₄ H ₈ | <i>t</i> -butyl, Aromatic <i>n,i</i> -alkyl (<i>n,i</i> >= 4), pentyl ketone |
| M-57 | C ₄ H ₉ | (<i>t</i>)-butyl-R |
| M-58 | C ₄ H ₁₀ | - |
| M-60 | CH ₃ COOH | Acetates |

If M^+ cannot be satisfactorily assigned, another peaks in the spectrum should be tried; if there is reason to believe that M^+ is not stable under EI conditions, other (softer) ionisation techniques (section 4) like CI, FAB, MALDI, electrospray etc. should be tried.

2.2. Fragmentation: General Considerations. The concept of charge location, i.e. that after ionization the charge will be located on the element with the lowest ionization potential, is helpful in understanding why molecular ions dissociate or fragment via a characteristic pattern. As already discussed in section 1.2 and Figure 1.6, the energy levels of the valence electrons in organic molecule are such that the probability of electron release during ionisation decreases in the order $n > \pi > \sigma$. Besides this idea of charge location, the structure of the molecule plays an essential role in dissociation. One of the most important underlying driving forces in dissociation/fragmentation is the formation of the most stable cation fragment (with a paired electron system, an even-electron ion) from the molecular radical cation (a system with an unpaired electron, or odd-electron ion).

A neutral, non-ionised compound has an even number of electrons. Ions can have either an even or an odd number of electrons. Calculating the unsaturation D (Eq.18) for an elemental composition gives the electronic configuration. This can be used to identify molecular ions, fragment ions and ions resulting from rearrangement reactions.

EI (Electron Impact) produces odd electron (OE) ions because one electron is lost during ionization. Fragmentation in EI usually occurs through the loss of a radical so that most fragment ions will have an even-electron configuration. Odd electron fragments usually result from rearrangement reactions and therefore it is useful to identify important odd-electron ions in a mass spectrum.

Soft ionization methods like FAB, CI, FD or Electrospray often produce species like $[M+H]^+$ or $[M+Na]^+$ that have an even-electron (EE) configuration. These considerations can help to limit the number of possible elemental compositions assigned to an ion. Looking for molecular ions in EI limits the search to odd-electron compositions whereas in FAB ($[M+H]^+$) the search is limited to even-electron ions.

For organic molecules without nitrogen (or with an even number of nitrogens) the OE molecular ion is expected to have an even mass in EI, while the EE fragments have an odd mass. For molecules and fragments with an odd number of nitrogens it is the other way around (Table 2.2). The EE molecular ion generated in CI has an odd mass, except when it contains an odd number of nitrogens.

Table 2.2. Odd and even masses and electrons in molecular ions and fragments.

| | EI molecular ion | EI fragment (radical loss) | EI fragment (neutral loss) | CI molecular ion |
|---------------|------------------|---|---|------------------|
| No N | Even mass, OE | Odd mass, EE | Even mass, OE | Odd mass, EE |
| Odd no. of N | Odd mass, OE | Even mass, EE (if N lost: Odd mass, EE) | Odd mass, OE (if N lost: Even mass, OE) | Even mass, EE |
| Even no. of N | Even mass, OE | Odd mass, EE | Even mass, OE | Odd mass, EE |

Important factors for fragmentation are:

- Energy of the molecular ion and the fragments formed from it
- Stability of the bonds in the ions
- For rearrangements: steric factors.

It is easier to move an H than a whole group

d) Stability of the formed ions or neutral particles
resonance stabilization such as in an acylium ion

An important guideline for the direction of fragmentation is Stevenson's Rule, which states that:

When cleaving a bond the positive charge preferably will end at the fragment with the lowest ionisation potential.

In practice this means that: Upon dissociation of $AB^+ \cdot \rightarrow A^+ + B\cdot$ or $A\cdot + B^+$, A^+ will be formed if it has the lower ionization energy, and that in branched radical cations, the largest group is preferentially lost. The easiest way to explain this is shown in Fig. 2.1. From this picture it is obvious that the reaction with the lowest AP, the formation of A^+ , is the process where the charge remains on the fragment with the lowest IP, ionisation potential ($I_A < I_B$). This is in agreement with the rule.

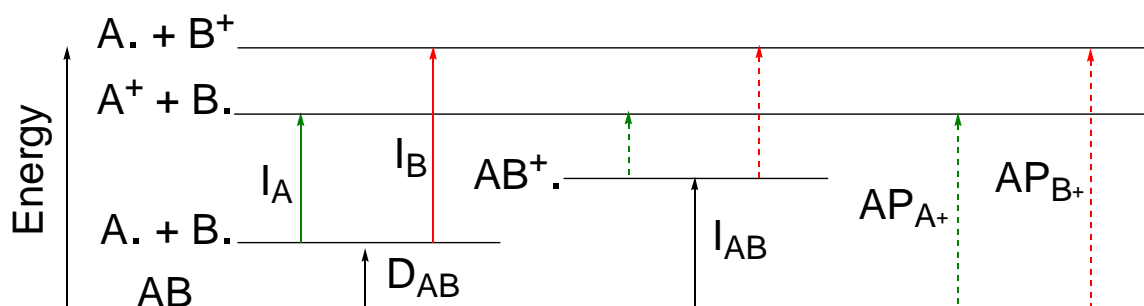


Figure 2.1. Illustration of Stevenson's Rule, with Ionization Energy (I), the energy required to convert radical into cation; Dissociation Energy (D), energy for homolytic dissociation into radicals, and Appearance Potential (AP), energy required for cation to appear in MS.

An example is the fragmentation of butyl methyl ether, which gives $CH_2=OCH_3^+$ as the most important fragment.

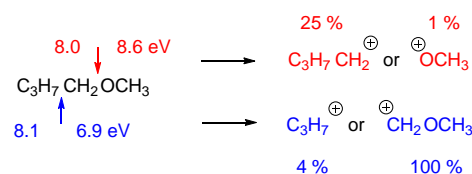
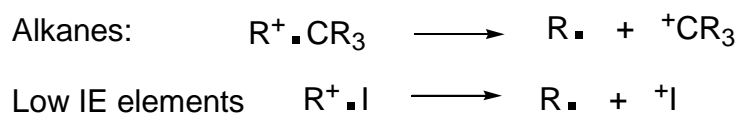


Figure 2.2. Fragmentation of butyl methyl ether in EI

2.3. Mechanisms of Fragmentation. Four fundamentally different mechanisms of fragmentation can be distinguished (MacLafferty & Tureček, 1993):

1) Sigma electron ionisation (σ): Apart from bonds between carbon and an element with extremely low ionisation energy, such as iodine, this occurs most likely in compounds that do not have any n or π electrons, such as alkanes, for which it is the only ionisation mechanism (Fig. 2.3).

Figure 2.3. Fragmentation following sigma electron ionisation.



It is observed that non-linear alkanes have a tendency to fragment near the branching points. In order to be able to predict at which site an alkane will fragment it is good to remind oneself of the relative stabilities of organic radicals and cations. There are parallels in the stabilities

series when alkyl groups are considered separately from other (benzyl, allyl, vinyl, phenyl) group (Fig. 2.4, left) and the energy required to convert radical into cation (ionisation energy) increases from tBu via the less branched alkyls, benzyl and allyl to vinyl, methyl, phenyl, and H (Fig. 2.4, right).

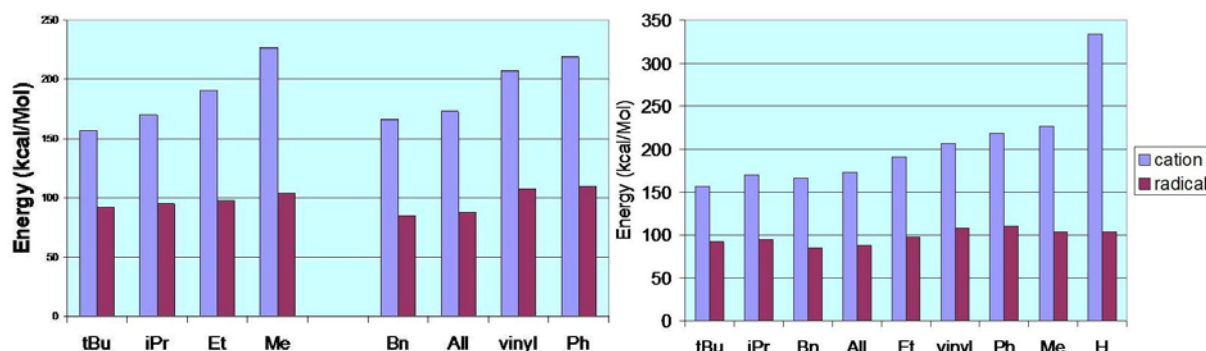


Figure 2.4. (Morrison & Boyd, Organic Chemistry, 6th ed. 1992). Enthalpies of cation (blue bars, heterolytic dissociation of R-Cl) and radical (red bars, homolytic dissociation of R-H) formation; left, alkyl and other radicals; right, increasing enthalpy of formation of cation from radical (ionisation potential).

If Stevenson's rule were generally applicable, one might expect in the example of Figure 2.5 the formation of the t-butyl cation (which has the lowest ionisation potential), which would be accompanied by that of a hydrogen radical, to dominate the mass spectrum for fragmentation around the branching point, but that is not what happens in reality. In fact the balance of the energies of formation of the cations and radicals involved generally leads to the outcome that, opposed to Stevenson's rule, the largest fragment is lost. In the example of Figure 2.5, this means that formation of the (secondary cation) fragment is observed, in which the t-butyl radical is lost.

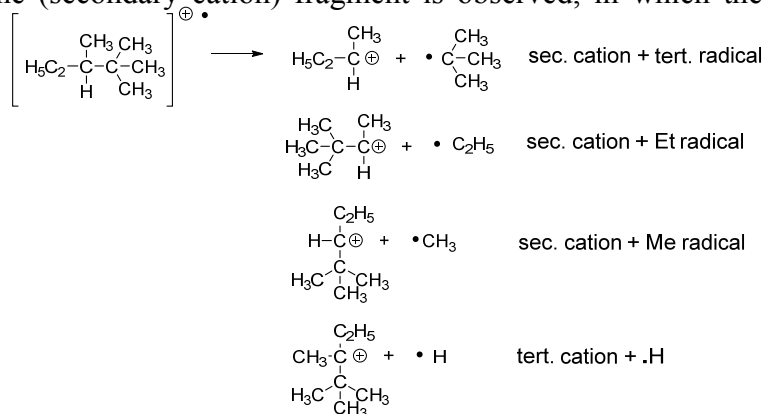


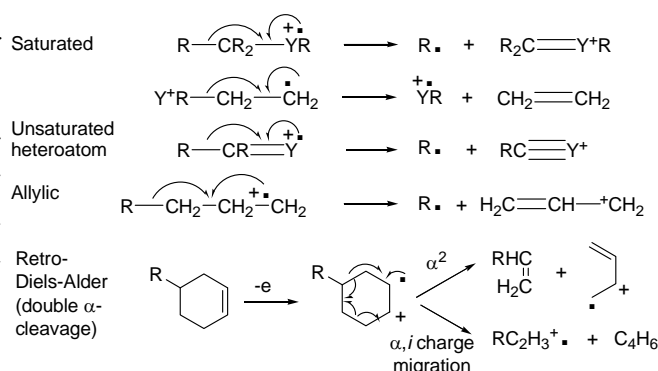
Figure 2.5. Fragmentation possibilities for a heavily branched alkane. The possibility at the top would be preferred.

2) Radical site initiation (alpha cleavage, α), homolytic dissociation.

The initial ionisation takes place by removing an electron from the non-bonding orbital of a heteroatom X. If the heteroatom X is not very electronegative (alpha cleavage favoured for $\text{N} > \text{S}$, $\text{R} > \text{O}$, $\pi > \text{Cl}$, $\text{Br} > \text{H}$) it can give up the unpaired electron and combine it with another from another bond (α) to the attached carbon to form an additional bond to this attached carbon; the other bond to this carbon, from which an electron is removed, is broken (Figure 2.6). The site of the radical is moved, while that of the positive charge is retained. In case of multiple possibilities for alpha fragmentation, the loss of largest alkyl group is favoured. Alpha cleavage can also start from a primary radical which results from an rH step (see below) as in a MacLafferty rearrangement, leading to the neutral loss of a (substituted) alkene, or on a heteroatom already connected by C by a double bond, in which case a triple bond is formed.

Figure 2.6. Fragmentation by alpha cleavage.

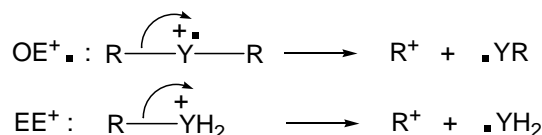
It can also be initialised by ionisation of a π electron, giving an allylic cation for a linear molecule after a single alpha cleavage, or loss of a (substituted) alkene after two alpha cleavages for cyclohexene in a so-called 'Retro Diels-Alder' fragmentation.



3) Charge site initiation (inductive effect, *i*), heterolytic dissociation.

This type of fragmentation can start by ionisation through the removal of a non-bonding electron from a heteroatom X, as in the case of alpha cleavage, but also in a EE fragment ion which bears a positive charge on X. If X is a relatively electronegative element (charge site initiation favoured by Cl, Br, NO₂ > O, S >> N, C), it will draw to itself the electron pair from a bond to carbon, which is then cleaved. The charge will end up on the fragment that does not contain X. In case of multiple possibilities, the most stable cation will be formed.

Figure 2.7. Fragmentation by charge site initiation.



4) Rearrangements (*r*):

In rearrangements, a group (typically H, *r*H) is transferred from another place in the molecule with an electron to the site of initial ionisation (Y).

This can occur in a 6-membered ring transition state as in the MacLafferty rearrangement for an unsaturated receptor (Figure 2.8). The final outcome depends on whether the new OE ion reacts by homolytic dissociation at the radical site (charge retention) or heterolytic dissociation at the charge site (charge migration).

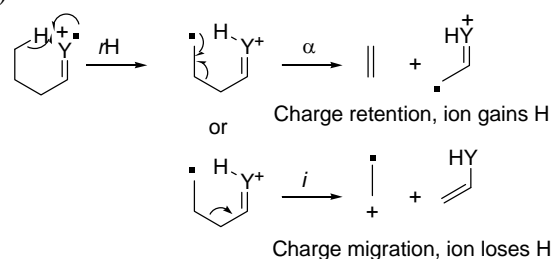


Figure 2.8. Rearrangements with H transfer and charge retention or migration.

In the so-called 'onium' reaction (Figure 2.9), which applies to any cation carrying a positive charge on a heteroatom Y (Y can be O (oxonium), N (nitronium), S (sulfonium) etc.) the rearrangement occurs with transfer of H to a saturated receptor site, and there is no strict requirement for a 6-membered ring transition state; the H can be transferred from anywhere in an alkyl chain to the heteroatom of initial ionization.

In OE ions, H is transferred with an electron, and the resulting alkyl radical moiety reacts to be removed from the radical cation as a neutral loss (alpha cleavage α or rearrangement with displacement *rd*); in EE ions, H is transferred with a pair of electrons, and the resulting alkyl cation is eliminated from the cation as a neutral loss (rearrangement with elimination *re*).

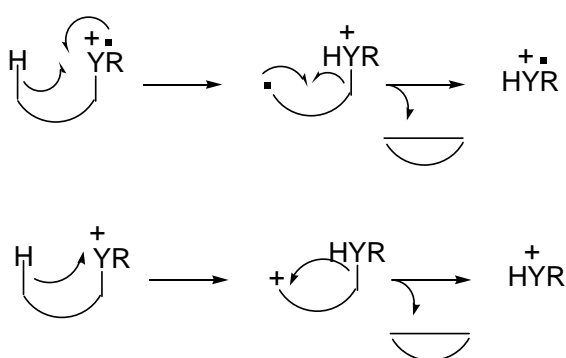


Figure 2.9. Onium reaction with *rd* or *re*.

2.4. Homolytic dissociation. Homolytic dissociation or alpha-cleavage means the cleavage of a bond X-Y, where one of the binding electrons is paired with the free electron on element Z adjacent to either X or Z. A so called one-electron-shift.

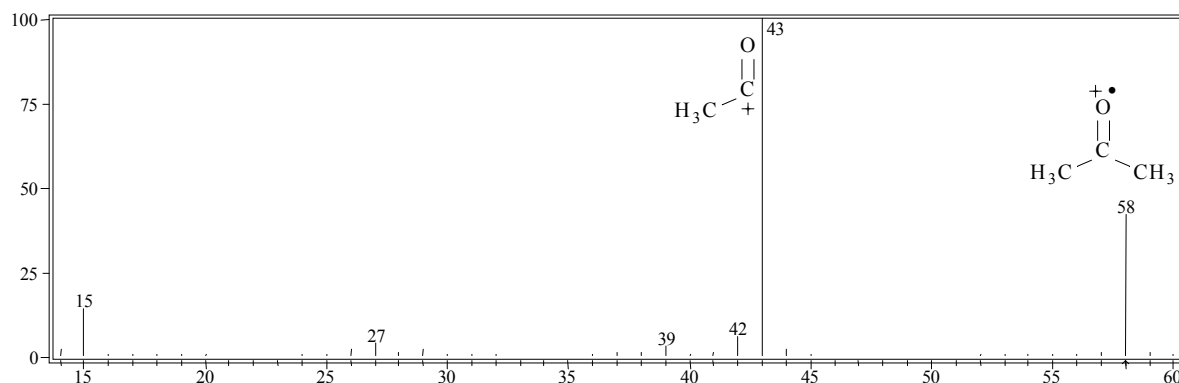


Figure 2.10: Mass spectrum of acetone

An example of homolytic dissociation is the dissociation of the molecular ion of acetone (Figure 2.10). Due to charge location this molecular ion will miss a n-electron and through homolytic dissociation it will lose a methyl-radical as confirmed by the mass spectrum (Figure 2.11).

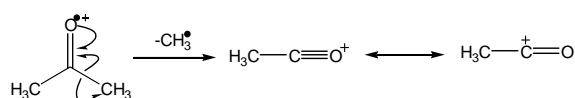
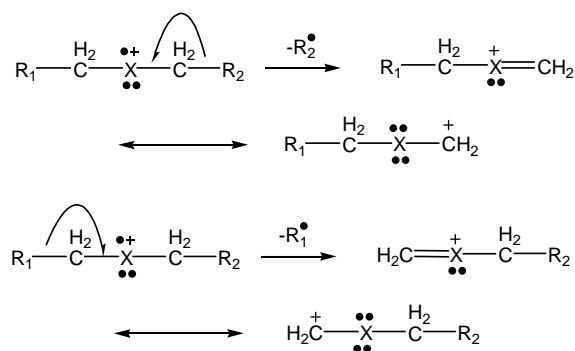


Figure 2.11: Loss of a methyl-radical by acetone.

With asymmetric molecules, there are more possible ways for homolytic dissociation (Fig. 2.12). One rule here is that the fragment resulting from the dissociation of the bigger radical has a higher intensity than the fragment resulting from the opposite. This only if it is possible to compare the dissociated radicals as in the example. This is caused by the fact that the biggest radical can absorb more vibrational and rotational energy than the smaller radical can. In the first, the remaining ion will be less tending to further dissociation than the ion in the second case. Several examples of competitive homolytic dissociation exist and in the following, five will be presented.



$\text{R}_1, \text{R}_2 = \text{H, alkyl}; \text{X} = \text{O, S, N etc.}$

Figure 2.12: Competitive homolytic dissociation.

2-Butanol

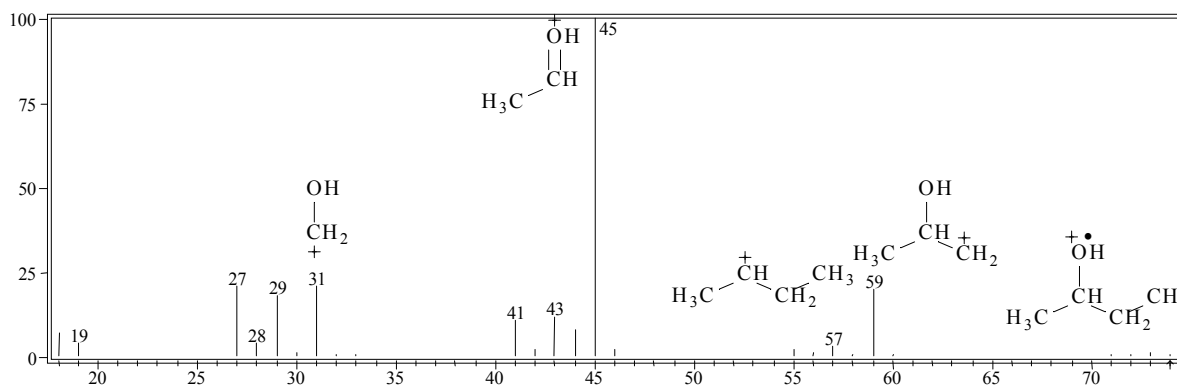


Figure 2.13: Mass spectrum of 2-butanol.

From the molecular ion we expect, in decreasing order, the dissociation of an ethyl-radical, a methyl-radical or a hydrogen atom (Fig. 2.13).

2-methyl-2-propanol

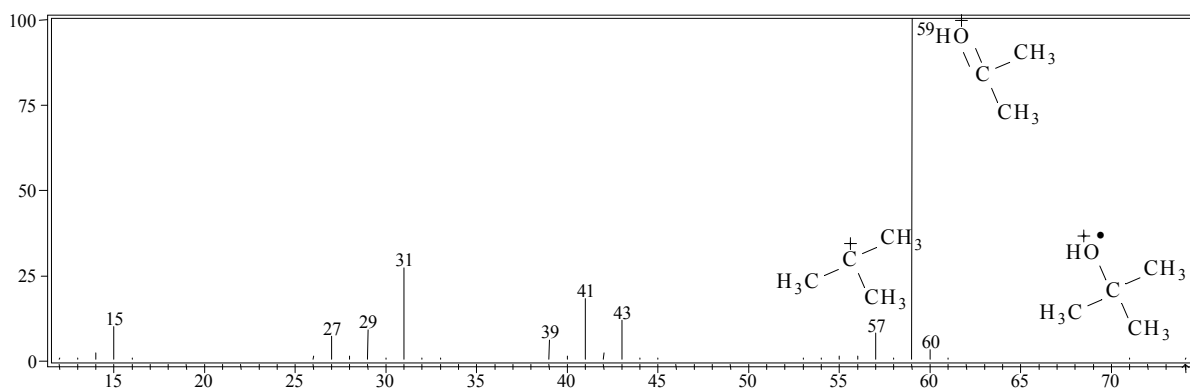


Figure 2.14: Mass spectrum of 2-methyl-2-propanol (t -butanol).

The spectrum (Figure 2.14) of the isomer of 2-butanol, 2-methyl-2-propanol or t -butanol, differs drastically from the former. This is because through α -cleavage from t -butanol only methyl-radicals can be split off.

N-ethyl-*n*-propylamine and *N*-(*tert*-butyl)-*N*-methylamine

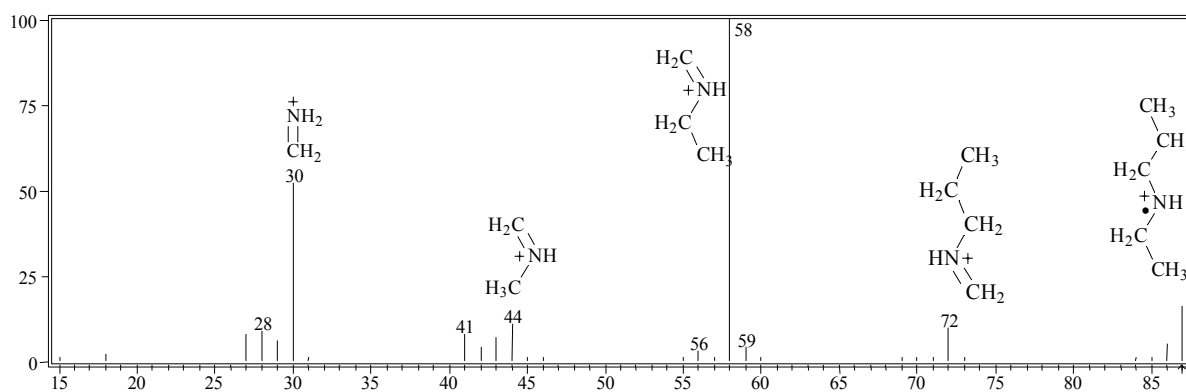


Figure 2.15: Mass spectrum of *N*-ethyl-1-Propanamine

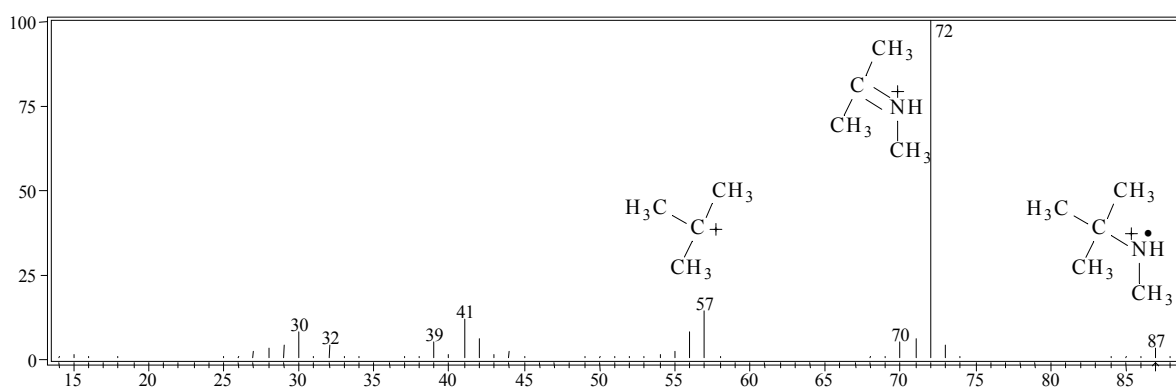


Figure 2.16: Mass spectrum of *N*-*tert*-Butylmethylamine.

For these compounds the same applies as for 2-butanol. Here also, in descending order, an ethyl-radical, a methyl-radical or a hydrogen atom is split off.

Ethylbenzene. Here one expects the molecular ion to miss a π -electron resulting in the dissociation of a methyl-radical or a hydrogen atom. Elaborate investigation has proven that the formed ions (m/z 91 and m/z 105) do possess a tropylium and a methyl-tropylium structure (Figure 2.17). These ions are very stable because they obey *The Hückel* rule for $(4n+2)$ conjugated π -electrons aromatic systems. They both have 6 conjugated π -electrons.

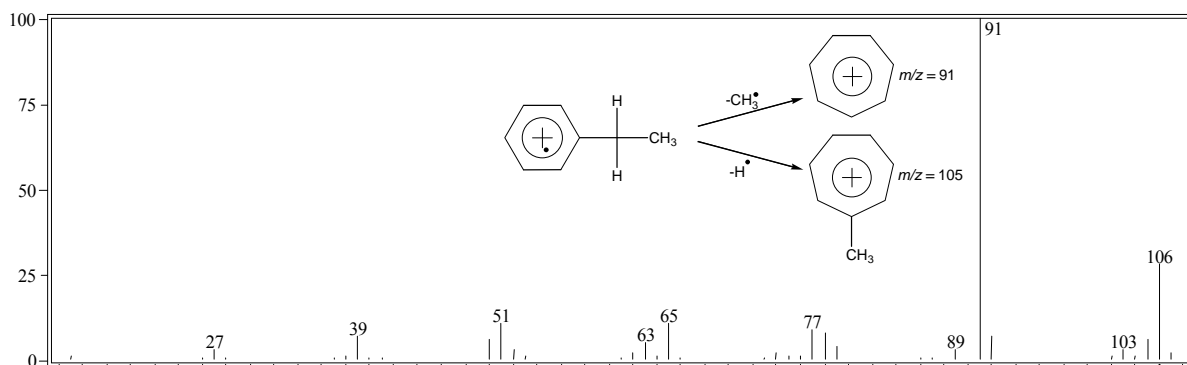
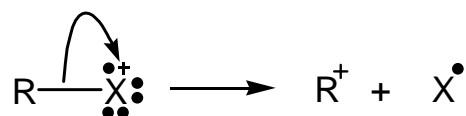


Figure 2.17: Mass spectrum and partial fragmentation scheme of ethylbenzene.

2.5. Heterolytic dissociation. Heterocyclic cleavage of a bond R-X means that both electrons of the bond are either transferred to fragment X or fragment R (Figure 2.18). The spectra of chloro-ethane and bromo-ethane (below) serve as an example. Here the base-peak is found at mass 29 due to heterolytic cleavage.



X = Cl, Br, I or another stable radical

Figure 2.18: Heterolytic dissociation

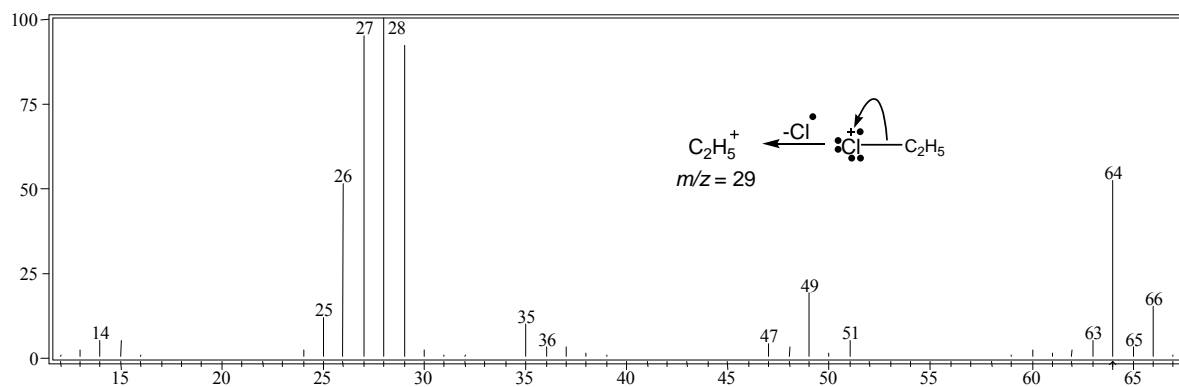


Figure 2.19: Mass spectrum of chloroethane.

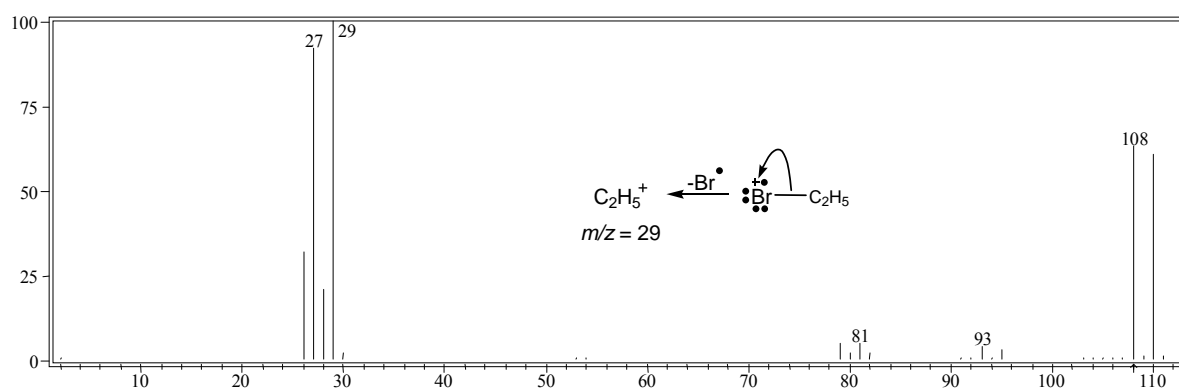


Figure 2.20: Mass spectrum of bromoethane.

This heterolytic cleavage also appears with ethers and thioethers. In the mass spectrum of dimethyl ether besides the base-peak at m/z 45, formed by α -cleavage, also a strong peak at m/z 15 appears.

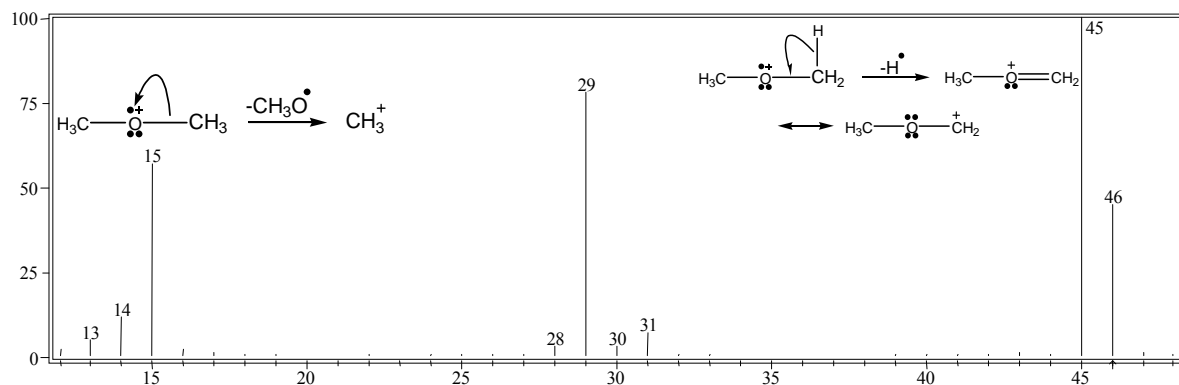


Figure 2.21: Mass spectrum of dimethyl ether.

2.6. Rearrangements

The McLafferty rearrangement. When we compare the mass spectra of 3-methyl-2-butanone and 2-pentanone the biggest difference is the presence of peak at m/z 58 in the latter (Figs. 2.22, 2.23).

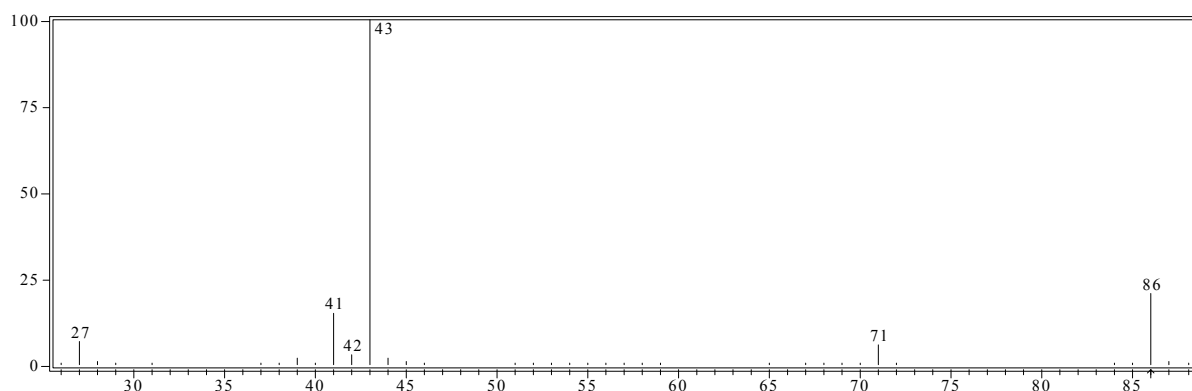


Figure 2.22: Mass spectrum of 3-methyl-2-butanone.

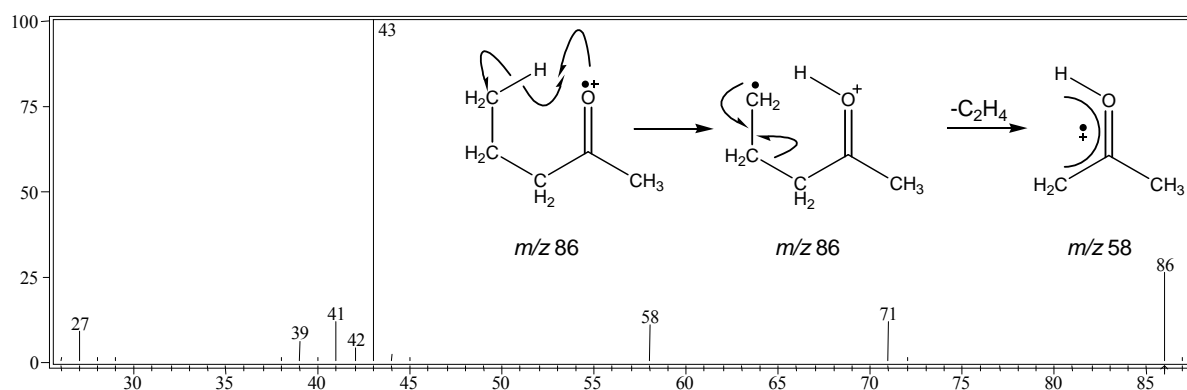


Figure 2.23: Mass spectrum of 2-pentanone.

The peaks at m/z 71 and m/z 43 in both cases are explained easily by α -cleavage. The most remarkable about m/z 58 is that it has an **even** mass. This only occurs if there are two bonds broken. When breaking 1 single bond in an ordinary compound containing C, H and O, always fragments are formed with an **odd** mass. McLafferty showed that the fragment at m/z 58 is formed from the molecular-ion of 2-pentanone in the way shown in Fig. 2.22. In this mechanism the γ H atom (6-position) moves to the oxygen-atom in the 1 position, followed by homolytic cleavage of the C_α - C_β -bond leading to the elimination of ethene. This reaction only occurs in the presence of a γ H-atom, is quite common and also appears in compounds with structures similar to the ones in Figs. 2.24 and 2.25.

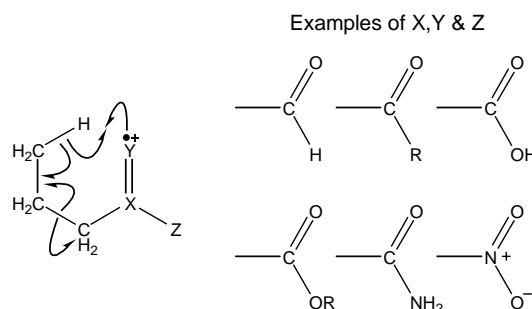


Figure 2.24: Examples of the McLafferty rearrangement.

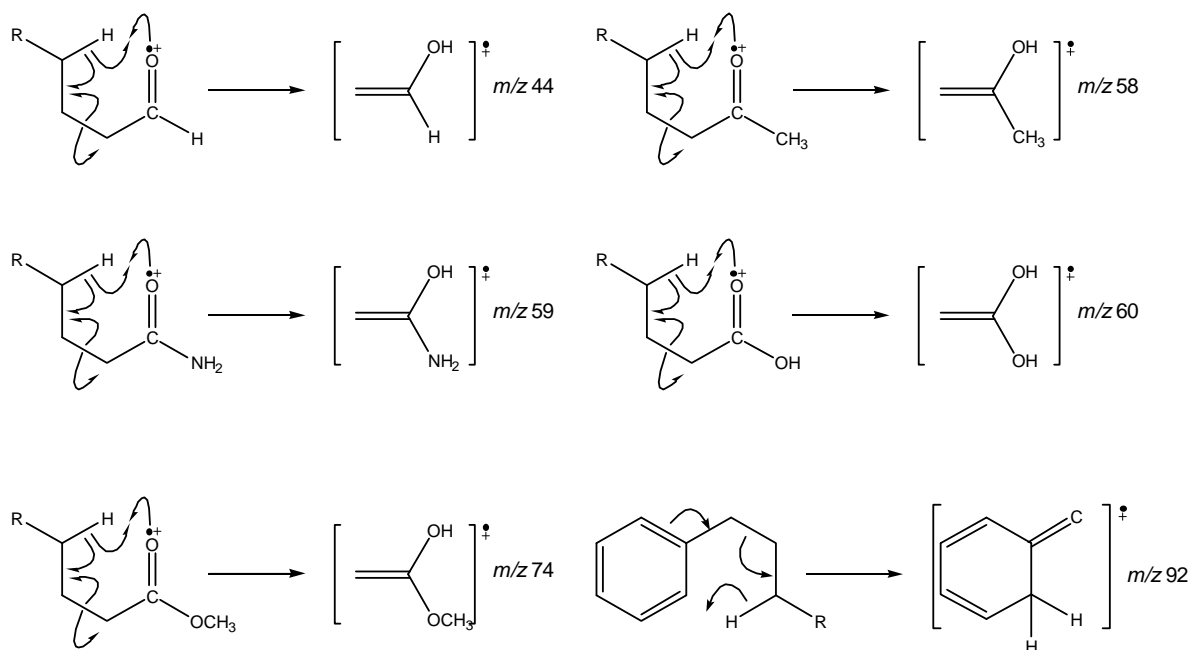


Figure 2.25: Some characteristic fragments formed in the McLafferty rearrangement.

The retro Diels-Alder reaction. In the Diels-Alder reaction, well-known from organic chemistry, a 6-membered ring with a single double bond is formed in a so-called pericyclic reaction, i.e. by concerted movement of electron pairs. With radical-ions containing such an unsaturated ring this reaction can easily proceed in the opposite direction and is called the retro Diels-Alder, even though the fragmentation is obviously not a pericyclic reaction. The fragment that will bear the charge depends on the nature of R. When R=H the intensity of [2] will be smaller than 5%. When R=C₆H₅ [2] will be the base-peak (100%) in the spectrum. In general both fragments will be found as can be seen in the example of 2-norbornene (Fig. 2.27).

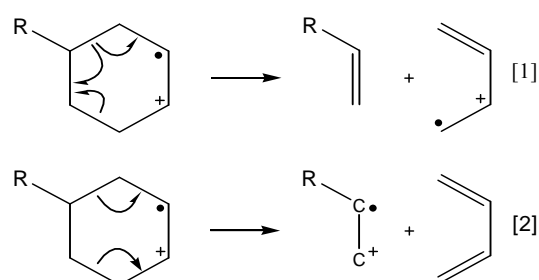


Figure 2.26: retro Diels-Alder mechanism.

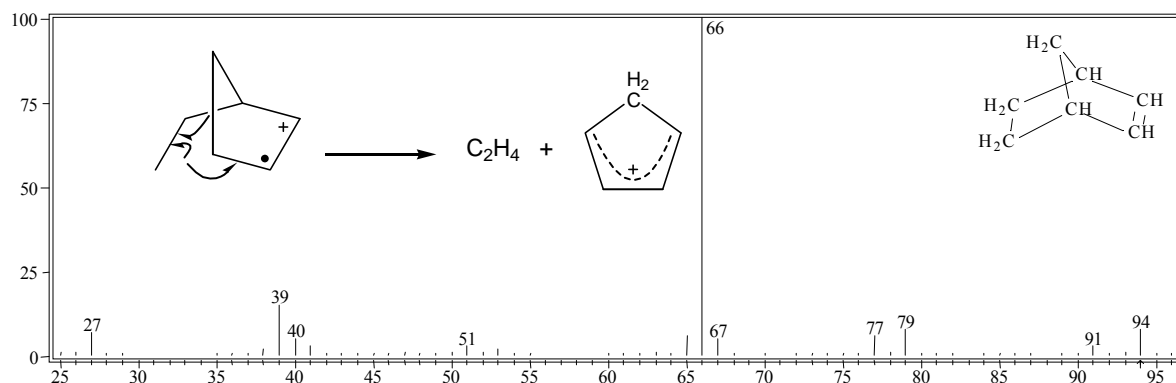
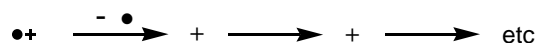
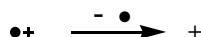


Figure 2.27: Mass spectrum and fragmentation of 2-Norbornene.

2.7. Further dissociation of fragment ions. The further fragmentation of the primarily formed fragment ions is not easily explained. We will pay attention to the most important cases only. First of all it has to be mentioned that EE cations are formed by both homolytic as well as heterolytic cleavage of the molecular OE radical-cation (see 2.1-2.4). This EE cation cannot lose radicals on further dissociation, but only neutral fragments. In shorthand:



The transition:



is called a “simple cleavage” transition.

Within the McLafferty rearrangement (2.6) from the molecular ion a neutral fragment is split off and hence the formed fragment is a radical cation. On further dissociation this will lose a radical first so that a cation is formed.

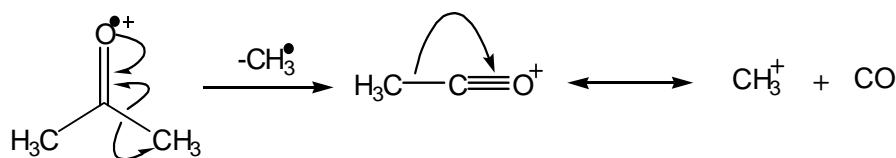


In this case the first transition:

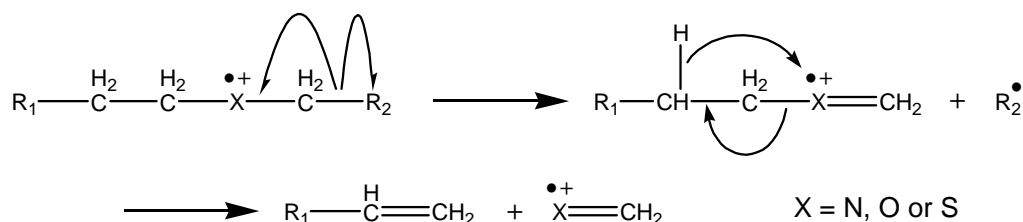


Is called a “rearrangement reaction”. Some examples of dissociations of primary formed ions will be given hereafter.

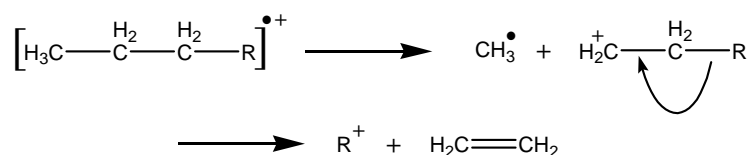
Loss of CO from acylium ions. From the molecular ion from acetone through homolytic cleavage an acylium ion is formed. This acylium ion can split off carbon monoxide through heterolytic cleavage.



Loss of alkenes from ethers, alcohols etc. After a homolytic cleavage of the molecular ion ether, alcohols, amines etc. can decompose further through split off of an alkene:



Alkanes also may lose alkenes, but without a proton transfer:



Formation of ion-series. As stated above, alkanes can decompose by losing ethene, after which an R^+ -ion remains. These R^+ -ions themselves decompose in the same way, resulting in other R'^+ -ions. These R^+ -ions and R'^+ -ions also might result from other reactions, e.g. through rearrangement reactions or directly from the molecular ion ($\text{M}^{+\bullet}$). Through all these possible reactions a series of $[\text{C}_n\text{H}_{2n+1}]^+$ -ions is formed. The maximum intensity for this series is found for $n=3$ (m/z 43) or $n=4$ (m/z 57). The series itself is characteristic for alkanes or compounds with a long alkane moiety. This kind of characteristic ion-series is also found with other classes of compounds. Some of the more important are listed in the next Table 2.3.

Table 2.3. Characteristic Ion Series

| Compound | Formula | m/z values | | | | |
|-----------------------|---------------------------------------|------------|----|-------|-------|-------|
| Alkanes | $\text{C}_n\text{H}_{2n+1}^+$ | 29 | 43 | 57 | 71 | 85 |
| Aldehydes, ketones | $\text{C}_n\text{H}_{2n-1}\text{O}^+$ | 29 | 43 | 57 | 71 | 85 |
| Amines | $\text{C}_n\text{H}_{2n+2}\text{N}^+$ | 30 | 44 | 58 | 72 | 86 |
| Ethers, alcohols | $\text{C}_n\text{H}_{2n+2}\text{O}^+$ | 31 | 45 | 59 | 73 | 87 |
| Sulfides | $\text{C}_n\text{H}_{2n+1}\text{S}^+$ | 47 | 61 | 75 | 89 | |
| Chloro-alkanes | $\text{C}_n\text{H}_{2n}\text{Cl}^+$ | 49 | 63 | 77 | 91 | |
| Alkenes, cylo-alkanes | $\text{C}_n\text{H}_{2n}^+$ | 28 | 42 | 56 | 70 | 84 |
| Aromatics | $\text{C}_n\text{H}_{<n}^+$ | 38 | 39 | 50-51 | 63-65 | 75-78 |

3. Ion Separation in Mass Spectrometry

3.1. General remarks. There are several ways to separate ions with different mass/charge ratios, e.g. magnetic sector analyzers, quadrupole mass filters, quadrupole ion traps, time-of-flight analyzers and ion cyclotron-resonance instruments. The first two types presently account for the great majority of instruments used in organic chemistry. Ideally, when separating, it is possible to distinguish between ions with very little difference in mass/charge ratio while maintaining a high flow of ions. These conditions are not in agreement and a compromise should be reached. For some applications a nominal mass discrimination will do, for other applications a much higher resolving power is needed. For example when one needs to distinguish between ions C_2H_4^+ , CH_2N^+ , N_2^+ and CO^+ (with respective masses of 28.031, 28.019, 28.006 and 27.995 amu) a resolving power of 0.01 mass units is needed. The main differences in mass-spectrometers are encountered in the way ions are separated.

3.2. Sector Instruments

'Single' focussing separation by magnetic deflection.

Separation in this way is effected by the application of a magnetic field perpendicular to the motion of the ions leaving the ion-source. Deflections of about 30 to 180 degrees are achieved (Figure 1.1). The trajectory of the ion follows from the applied forces: The Lorentz force and the centrifugal force. The Lorentz force F_L (Fig. 3.1) is given by:

$$F_L = Bzev \quad (1)$$

Where B is the strength of the magnetic field, z the amount of charges, e the charge of one electron and v is the velocity. When traversing a radial path of curvature r through a magnetic field B this force equals the angular momentum:

$$F_c = \frac{mv^2}{r_{ion}} \quad (2)$$

The energy of the ion is expressed as:

$$E_{kin} = zeU = \frac{1}{2}mv^2 \quad (3)$$

Where U is the accelerating voltage. For an ion, that reaches the detector (1) equals (2), hence:

$$Bzev = \frac{mv^2}{r_{ion}} \quad (4)$$

Substitution of (4) in (3) and rearranging gives:

$$\frac{m}{z} = \frac{B^2 r^2 e}{2U} \quad (5)$$

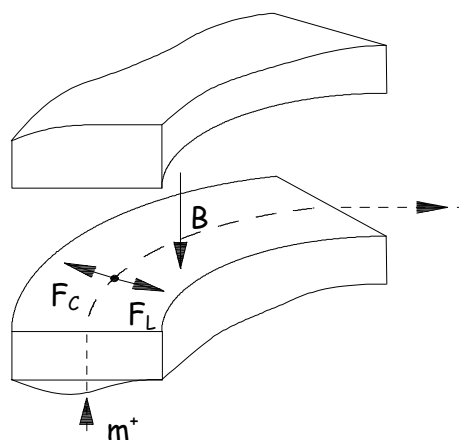


Figure 3.1: Magnetic analyzer.

Note that the rearrangement of (4) to $mv = Bzer$ demonstrates the fact that a magnetic sector is a momentum analyzer rather than a mass analyzer as is commonly assumed. Expressed in practical units the atomic mass (M) of a singly charged ion is given by:

$$M = 4.83 \cdot 10^3 \cdot \frac{B^2 r^2}{U} \quad (6)$$

Where r is in centimeters, B is in tesla ($1 \text{ tesla} = 10^4 \text{ gauss}$), and U is in Volts. For example, a maximum field strength of 2 tesla gives a maximum mass just over 10000 Dalton for an instrument of 65 cm radius operating at an accelerating voltage of 8000V.

Equation (5) shows that by varying either B or U ions of different m/z ratio, separated by the magnetic field, can be made to reach the collector. The most common form of mass scan is the exponential magnet scan, downward in mass. This has the advantage of producing mass-spectral peaks of constant width. The equations appropriate to this form of scan are:

$$m = m_0 \cdot e^{-kt} \quad (7)$$

and

$$t_p = \frac{t_{10}}{2.303 \cdot R} \quad (8)$$

Where m_0 is the starting mass at time $t=0$. M is the mass registered at time t . t_p is the peak width between its 5% points. t_{10} is the time taken to scan one decade in mass (e.g. m/z 500 to 50) and R is the resolving power measured by the 10% valley definition. Scanning of the accelerating voltage U apparently has advantages because of speed and ease of control, however causes defocusing and loss of sensitivity and is therefore rarely used.

Double focusing separation. Because the magnetic sector separates on basis of momentum ions with little difference in translational energy are not focussed in the same point. The spread in translational energy of the ions formed in an electron-impact source limits the resolving power. In addition, source contamination leading to charging effects and contact potentials worsens this. Other ion sources like field desorption produce ions with an even larger spread in translational energy.

In a double focusing mass spectrometer, the ions are lead through a radial electrostatic field prior to magnetic separation. Therefore, only ions with the same kinetic energy are fed to the magnetic sector. In this way, the electrostatic analyzer (Fig. 3.2) acts as a 'source' and the combination of the two sectors can be designed to have velocity-focusing properties.

After acceleration, the ions possess a kinetic energy given by:

$$E_{kin} = zeU = \frac{1}{2}mv^2 \quad (3)$$

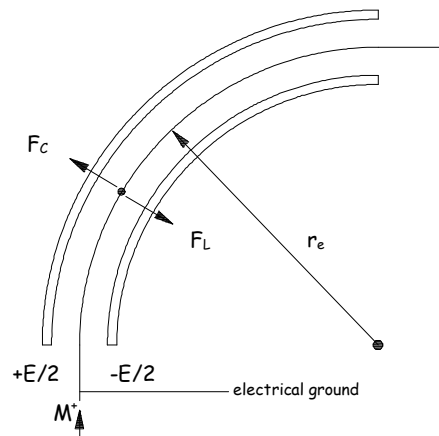


Figure 3.2: Electrostatic analyzer.

The centripetal force is given by:

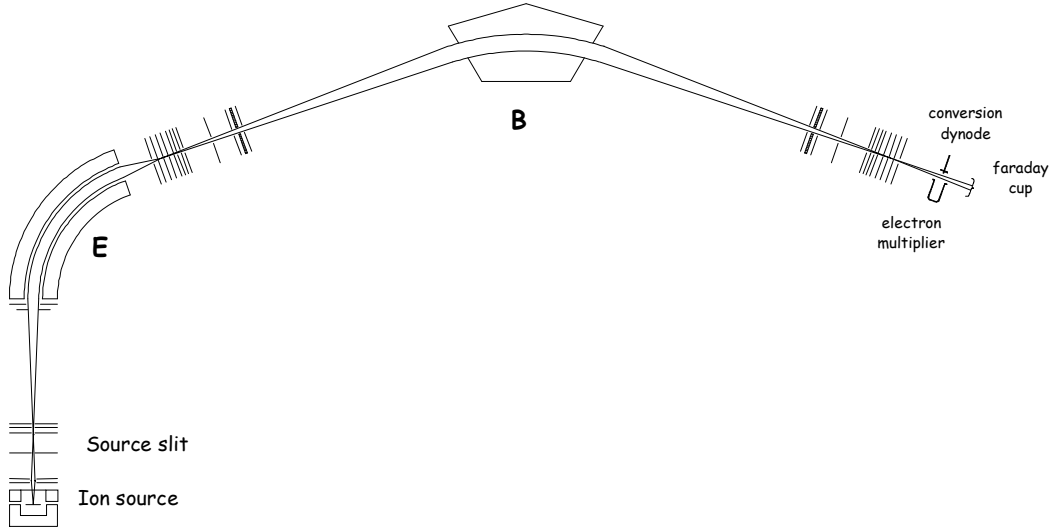


Figure 3.3. Scheme of a double-focusing magnetic sector instrument of Nier-Johnson geometry.

$$F_c = \frac{mv^2}{r_{ion}} \quad (2)$$

The electrostatic force is given by:

$$F_L = zeE \quad (9)$$

For ions leaving the electrostatic analyzer, (2) equals (9) so:

$$r_{ion} = \frac{mv^2}{zeE} = E_{kin} \cdot \frac{2}{zeE} \quad (10)$$

This shows that ions are separated by the electrostatic analyzer according to their kinetic energy. Substitution of (3) in (10) gives:

$$r_{ion} = 2 \cdot \frac{U}{E} \quad (11)$$

According to (11), the radius of an ion travelling through the electrostatic analyzer is independent of charge and mass.

A narrow slit placed in the image plane of an electrostatic sector can be used as ion source for a magnetic sector instrument. The energy filtering gives better resolution but gives loss of sensitivity due to the rejection of ions. By a proper choice in the combination of magnetic and electrostatic sectors, the velocity dispersion is equal and opposite in both sectors. The Nier-Johnson geometry as shown in Figure 3.3 is of this type and is one in which both mass and energy focusing occur at a single point. Most sector instruments intended for medium or high-

performance work in organic analysis are based on either conventional or reversed Nier-Johnson geometry.

Summary. Double-focussing magnetic sector mass analyzers are the 'classical' instruments against which other mass analyzers are compared. The characteristics are listed below.

- Classical shaped mass spectra
- Very high reproducibility
- Best quantitative performance of all mass spectrometer analyzers
- High resolution
- High sensitivity
- High dynamic range
- Linked scan MS/MS does not need another analyzer
- High energy CID (collision induced decay) MS/MS spectra are very reproducible

The limitations of sector instruments can be summarized as:

- Not well-suited for pulsed ionization methods (e.g. MALDI)
- Usually larger and higher costs both in purchase and maintenance than other mass analyzers
- Linked scanning MS/MS gives either limited precursor selectivity with unit product-ion resolution or nominal precursor selection with poor product-ion resolution

Applications

- All organic MS analysis methods
- Accurate mass measurements/peak-matching
- Quantitation
- Isotope ratio measurements

3.3. Quadrupole analyzer. The quadrupole mass filter consists of four parallel rods of hyperbolic or circular cross-section arranged symmetrically to a z-axis (Fig. 3.4). A voltage made up of a dc component U and a radio-frequency (r.f.) component $V_0 \cos(\omega t)$ is applied to adjacent rods. Opposite rods are electrically connected. Ions injected into the filter with a very small accelerating voltage, typically 10-20 V, are made to oscillate in the x and y directions by this field.

The parameters a and q are defined by:

$$a = \frac{8eU}{m r_0^2 \omega^2} \quad (12)$$

$$q = \frac{4eV_0}{m r_0^2 \omega^2} \quad (13)$$

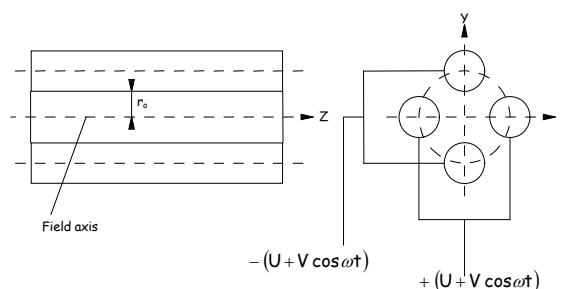


Figure 3.4: Schematic diagram of a quadrupole analyzer.

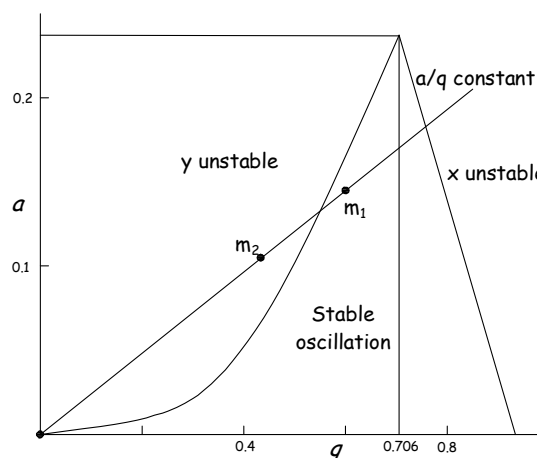


Figure 3.5: Stability diagram for a quadrupole analyzer.

In these equations $2r_0$ is the rod spacing and ω is the frequency of the r.f. voltage. For certain values of a and q the oscillations performed by the ions are stable, i.e. their amplitudes remain finite, but for other values of a and q these are unstable and the amplitude becomes infinite. The stability diagram, which is also known as Mathieu diagram (Figure 3.5), shows the values of a and q for which these conditions apply.

Ions with masses like m_1 fall into the stable oscillation area and will migrate towards the detector. Ions with masses like m_2 are outside the stable oscillation area and will go lost before they reach the detector and hence mass separation is achieved. Scanning of the mass spectrum is achieved by variation of U and V_0 while maintaining the ratio U/V_0 constant. The registered mass is proportional to V_0 so a linear scan of V_0 gives an easily linear calibrated mass spectrum.

Quadrupole instruments are deservedly popular because they are compact, robust, and relatively inexpensive and need little experience to operate. Compared to magnetic sector instruments there are two major advantages: ease of automated data control & handling and ease of interfacing with a variety of inlet systems.

Quadrupole analyzers do not have a large mass range nor a high resolution like sector instruments. They are, however, part of more sophisticated instruments as hybrid mass spectrometers and in tandem mass spectrometry. By application of other ionization techniques as electrospray, with multiple charged ions, the lack of mass range can be overcome.

Benefits of a quadrupole mass analyzer can be summarized as:

- Classical mass spectra
- Good reproducibility
- Relatively small and low-cost systems
- Low-energy collision-induced dissociation (CID) MS/MS spectra in triple quadrupole and hybrid mass spectrometers have an efficient conversion of precursor to product

Limitations of a quadrupole mass analyzer can be summarized as:

- Limited resolution
- Peak heights are variable as a function of the mass (mass discrimination). The peak height versus response must be 'tuned'.
- Not well suited for pulsed ionisation techniques.
- Low energy collision-induced dissociation (CID) MS/MS spectra in triple quadrupole and hybrid mass spectrometers depend strongly on energy, collision gas, pressure and other factors.

Applications of a quadrupole mass analyzer can be summarized as:

- Majority of benchtop GC/MS and LC/MS systems.
- Triple quadrupole MS/MS systems
- Sector / quadrupole hybrid MS/MS systems.

3.4. Ion trap. In Figure 3.6, a cross-section of an ion trap is shown. The three dimensional ion trap is a solid revolution of a quadrupole produced by rotation of the cross-section around the z -axis (Figure 3.4).

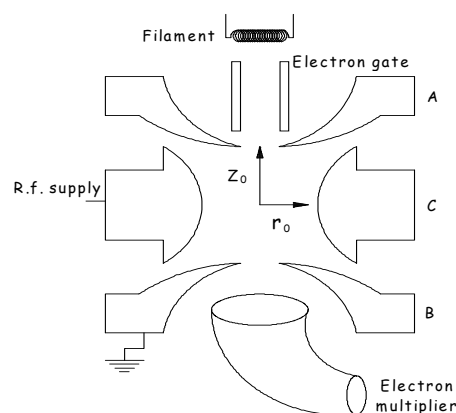


Figure 3.6: Schematic diagram of an ion trap.

The ion trap contains three cylindrically symmetrical electrodes: two end-caps, A and B, and a ring C. The ring electrode is fed with an r.f. voltage (V) and some times with an additional d.c. voltage (U) relative to the end-cap electrodes. Operating parameters a_z and q_z , analogue to the quadrupole, can be defined for the ion trap. In this case, r_0 is the internal radius of the ring electrode, about one cm, making an ion trap a small-scale device.

The use of an r.f. voltage causes rapid reversals of the field direction so the ions are alternately accelerated and decelerated in the axial (z) direction and *vice versa* in the radial direction. Regions of stable motion, in which ions are trapped in the cell, are described by a Mathieu diagram, as for a quadrupole mass filter (Figure 3.7).

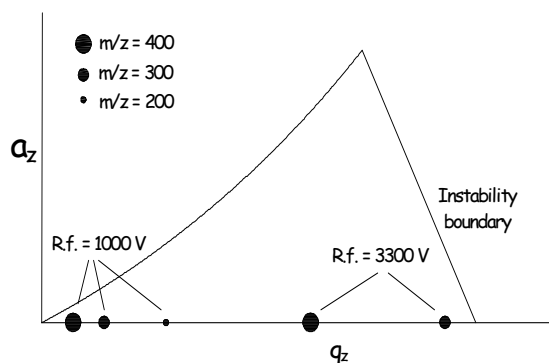


Figure 3.7: Stability diagram for an ion-trap.

Scanning mass analysis with an ion trap is known as the mass-selective instability mode. In this mode the r.f. frequency (about 1.1 MHz) and initial amplitude with a d.c. of zero are chosen so that all ions are stored with an m/z value greater than a threshold value. Thus, first ions are generated by the electron beam and trapped in the trapping field. After a short time, the electron beam is turned off and ions with an m/z value below the threshold are allowed to escape from the trap.

Now the threshold value of the trap is increased by increasing the amplitude of the r.f. voltage (Fig. 3.8) and the ions that become unstable will leave the trap through the holes in one of the end-caps and strike the detector.

A build-up of ion density within the trap can lead to space-charge effects, which will modify the electric fields within the trap. This can be overcome by the use of an automatic gain control (AGC). Here an initial ionization period is used to calculate the optimal ionization time, with no space-charge effects, for a second more extended ionization period. Following this second ionization period, the r.f. voltage is ramped to affect a mass scan as before.

The long storage times used in the ion-trap cause ion-molecule reactions like those in chemical ionization at much lower reagent gas pressure than commonly used in conventional high pressure sources. The significant trapping times also can lead to ion-molecule reactions involving analyte molecules which result in abnormal $(M+1)/M$ ratios under scan conditions. This only can be overcome to a certain degree by lowering the analyte concentration in the trap.

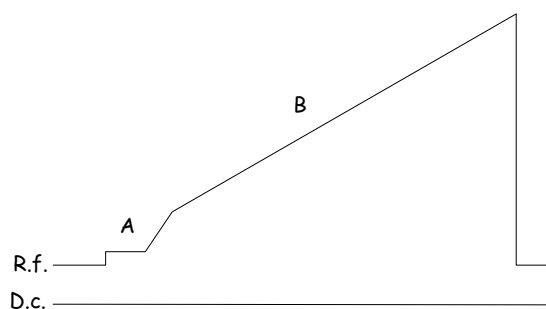


Figure 3.8: Mass-selective instability (period B).

Benefits of a quadrupole ion-trap mass analyzer can be summarized as:

- High sensitivity
- Multi-stage mass spectrometry (analogous to FTICR)
- Compact mass analyzer

Limitations of a quadrupole ion-trap mass analyzer can be summarized as:

- Poor Quantitation
- Very poor dynamic range (sometimes compensated by auto-ranging)
- Subject to space-charge effects and ion-molecule reactions
- Collision energy not well defined in CID MS/MS
- Many parameters comprise the experiment sequence that defines the quality of the mass spectrum (e.g. Excitation, trapping, detection conditions)

Applications of a quadrupole ion-trap mass analyzer can be summarized as:

- Benchtop GC/MS, LC/MS and MS/MS systems
- Target compound screening
- Ion chemistry

3.5. Time-of-flight analyzer. For an ion accelerated by a voltage V , the resulting velocity is proportional to the mass-to-charge ratio. In a time-of-flight (TOF) mass spectrometer (Figure 3.9), ions are separated on basis of the time t needed to travel a path L .

$$t = \sqrt{\frac{m}{2zeV}} \cdot L \quad (14)$$

For further reading on this topic see Guilhaus (1995). Ions of very high mass-to-charge (several hundreds of kD) may be recorded after an appropriate length of time.

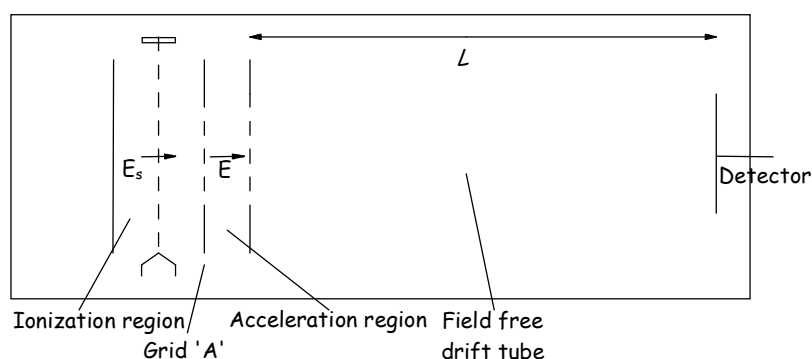


Figure 3.9: Schematic diagram of a time-of-flight instrument.

The potentially high sensitivity of a TOF instrument results from the high transmission due to the absence of beam defining slits and the temporal separation that in contrast to spatial separation does not direct ions away from the detector.

In contrast to continuous gas phase ionization processes, like electron impact ionization, TOF mass spectrometers are direct and simply compatible with ion formation from a surface, e.g. laser desorption (like in MALDI) and plasma desorption mass spectrometry. The pulsed character of these techniques provides a precisely defined ionization time and a small precisely defined ionization region, which is ideal for TOF-analysis.

Resolution in TOF-analysis may be improved by narrowing down the time-window and the ionization-region. Resolution also may be improved by application of a reflectron. The ions leaving the source of a time-of-flight mass spectrometer have neither exactly the same starting times nor exactly the same kinetic energies. Various time-of-flight mass spectrometer designs

have been developed to compensate for these differences. A reflectron (Figure 3.10) is an ion optic device in which ions pass through a mirror or reflectron and their flight is reversed. A linear-field reflectron allows ions with greater kinetic energies to penetrate deeper into the reflectron than ions with smaller kinetic energies. The deeper penetrating ions will travel a longer time to the detector opposite to the ions with a smaller energy. A reflectron thus decreases the spread in ion flight times for a given packet of ions and therefore improves the resolution of the TOF mass spectrometer.

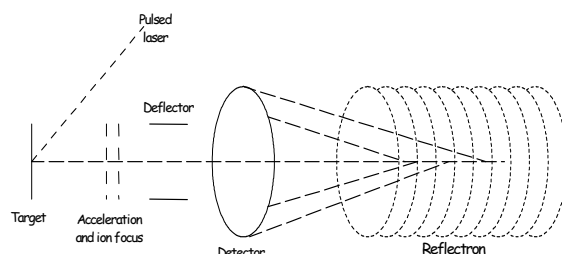


Figure 3.10: Schematic diagram of a reflectron.

Benefits of a time-of-flight (TOF) mass analyzer can be summarized as:

- Fastest available MS analyzer
- Well suited for pulsed ionization methods (majority of MALDI mass spectrometers is equipped with TOF)
- High ion transfer
- MS/MS information from post-source decay
- Highest practical mass range of all MS analyzers.

Limitations of a time-of-flight (TOF) mass analyzer can be summarized as:

- Requires pulsed ionization methods or beam switching
- Fast digitizers used in TOF may have limited dynamic range
- Limited precursor-ion selectivity for most MS/MS experiments

Applications of a time-of-flight (TOF) mass analyzer can be summarized as:

- Almost all MALDI (matrix assisted laser ionization) systems
- Very fast GC/MS systems

4. Ionization and Desorption

4.1. General Remarks. A mass spectrometer works by using magnetic and electric fields to exert forces on charged particles (*ions*) in a vacuum. Therefore, a compound must be charged or *ionized* to be analyzed by a mass spectrometer. As discussed in the Introduction/Basic Principles section, for *gas phase ionization* techniques such as Electron Impact, Chemical Ionization, and Negative Ion Chemical Ionization, 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 if they are to be analyzed by mass spectrometry. Although 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.

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*' methods such as field desorption and electrospray ionization tend to produce mass spectra with little or no fragmentation content.

4.2. Field Desorption and Ionization. These methods use very strong electric fields to extract ions from a substrate in vacuum. They provided one of the earliest means by which molecular ions could be generated from non-volatile and/or thermally unstable materials. Field ionization (FI) and field desorption (FD) can be distinguished by the way samples are supplied to the emitter. In FI, samples are introduced in the gas-phase from another inlet system. In FD the samples is coated on the emitter prior to analysis. The intense electric fields used in both FI and FD are produced with wire-based emitters. These emitters have multipoint micro-needles (dendrites, $\varnothing \sim 3\text{\AA}$) on their surface. The field strength at the tip of a micro-needle is given by:

$$F = V \cdot \left[\frac{(1-\alpha)}{\frac{1}{2} \cdot r \cdot \ln\left(\frac{4d}{r}\right)} + \frac{\alpha}{r} \right] \quad (19)$$

where V is the applied voltage, r is the radius of the micro-needle, d is the distance to the counter electrode and α is a shape factor (~ 0.0064). The energy transferred in FI and FD is only a fraction of an electronvolt, EI (~ 7 , <10) and CI (<5), so fragmentation is not or hardly introduced.

Field Desorption (FD). The sample is deposited onto the emitter that is biased to a high potential (several kilovolts). A current is passed through the emitter to heat up the filament. Mass spectra are acquired as the emitter current is gradually increased and the sample is evaporated from the emitter into the gas phase. The analyte molecules are ionized by electron tunneling at the tip of the emitter 'whiskers' (Figure 4.1, left). Characteristic positive ions produced are radical molecular ions and cation-attached species such as $[M+Na]^+$ and $[M-Na]^+$. The latter are probably produced during desorption by the attachment of trace alkali metal ions present in the analyte.

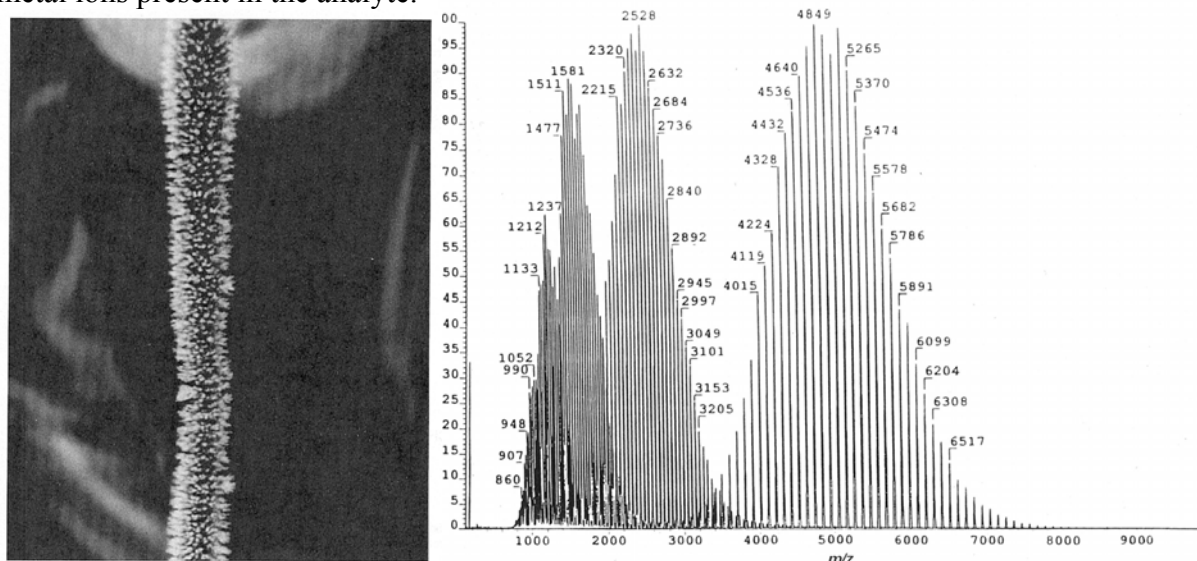


Figure 4.1. (left) Field Desorption whiskers, (right) FD spectrum of polystyrene revealing the presence of both polydispersity and charge clusters.

Figure 4.1, right, shows the FD mass spectrum of a polystyrene sample with an average molecular weight of approx. 5,100. In the m/z area ($z = 1$) around this average, the peaks are nicely separated by the weight of a styrene monomer, C_8H_8 (104). At lower m/z values, similar patterns, so-called charge clusters, are observed for the styrene polymer molecules that carry charges of +2, +3, +4, etc., in which the peaks are separated by the styrene monomer weight divided by z : $104/2$, $104/3$, $104/4$ etc.

Sample introduction

- Direct insertion probe.
- The sample is deposited onto the tip of the emitter by dipping the emitter into an analyte solution
- depositing the dissolved or suspended sample onto the emitter with a micro-syringe

Benefits

- simple mass spectra, typically one molecular or molecular-like ionic species per compound.
- little or no chemical background
- works well for small organic molecules, many organometallics, low molecular weight polymers and some petrochemical fractions

Limitations

- sensitive to alkali metal contamination and sample overloading
- emitter is relatively fragile
- relatively slow analysis as the emitter current is increased
- the sample must be thermally volatile to some extent to be desorbed

Mass range

- *Low moderate*, depends on the sample. Typically less than about 2,000 to 3,000 D.
- some examples have been recorded from ions with masses beyond 10,000 D.

Field Ionization (FI). The sample is evaporated from a direct insertion probe, gas chromatograph, or gas inlet (Figure 4.2). As the gas molecules pass near the emitter, they are ionized by electron tunneling.

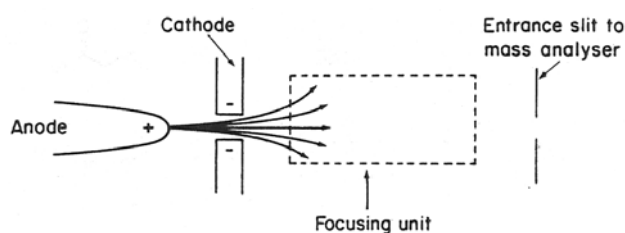


Figure 4.2. Field Ionisation (Barker).

Figure 4.3 shows a comparison of mass spectra obtained with EI, FI, and FD for xanthosine and glutamic acid. It can be seen that compared to EI, FI is the softer ionisation method, providing the molecular ion for both xanthosine and glutamic acid that cannot be observed in EI; for glutamic acid, the FD spectrum gives the molecular ion without fragmentation.

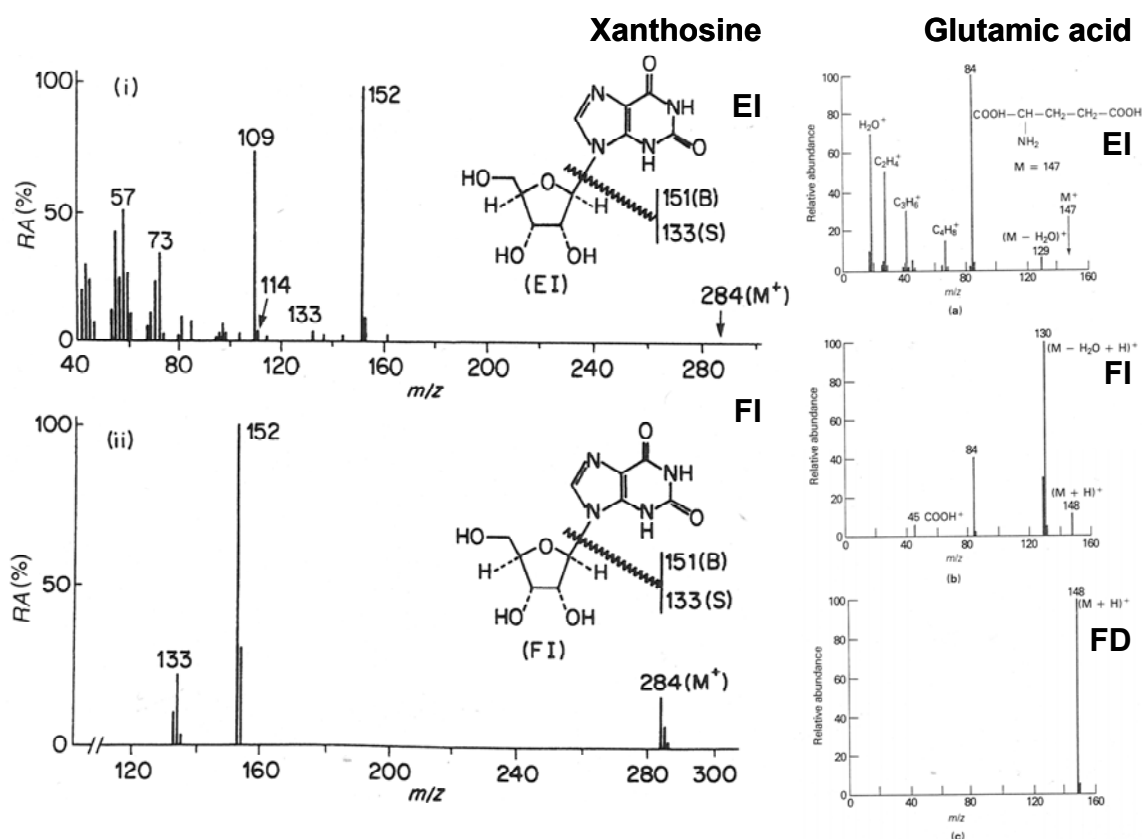


Figure 4.3. (left) EI and FI of xanthosine, (right) EI, FI, and FD of glutamic acid.

Sample introduction

- heated direct insertion probe
- gas inlet
- gas chromatograph

Benefits

- simple mass spectra, typically one molecular or molecular-like ionic species per compound.
- little or no chemical background
- works well for small organic molecules and some petrochemical fractions

Limitations

- The sample must be thermally volatile. Samples are introduced in the same way as for electron ionization (EI).

Mass range

- *Low* Typically less than 1000 D.

4.3. 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 (*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 organic mass spectrometry.

The *primary* particle beam is the bombarding particle beam, while the *secondary ions* are the ions produced from bombardment of the target.

Fast Atom Bombardment (FAB). The analyte is dissolved in a liquid matrix such as glycerol, thio-glycerol, *m*-nitrobenzyl alcohol, or diethanolamine and a small amount (about 1 microlitre) is placed on a target.

The target is bombarded with a fast atom beam (for example, 6-8 keV Xenon atoms), that desorbs molecular-like ions and fragments from the analyte (Figure 4.4). Cluster-ions from the liquid matrix are desorbed also, and produce a chemical background.

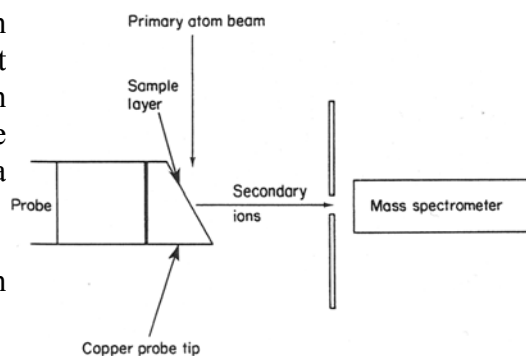


Figure 4.4. Desorption by Fast Atom Bombardment.

Sample introduction

- direct insertion probe
- LC/MS (*frit FAB* or *continuous-flow FAB*).

Benefits

- rapid
- simple
- relatively tolerant of variations in sampling
- good for a large variety of compounds
- strong ion currents -- good for high-resolution measurements

Limitations

- high chemical background defines detection limits
- may be difficult to distinguish low-molecular-weight compounds from chemical background
- analyte must be soluble in the liquid matrix
- no good for multiply charged compounds with more than 2 charges

Mass range

- *Moderate*: Typically ~300 D to about 6000 D.

Secondary Ion Mass Spectrometry (SIMS). *This discussion refers to dynamic SIMS.*

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.

The use of SIMS for moderate-size (3000-13,000 D) proteins and peptides has largely been supplanted by electrospray ionization.

Sample introduction

- Same as for FAB

Benefits

- Same as for FAB, except sensitivity is improved for higher masses (3000 to 13,000 Da).

Limitations

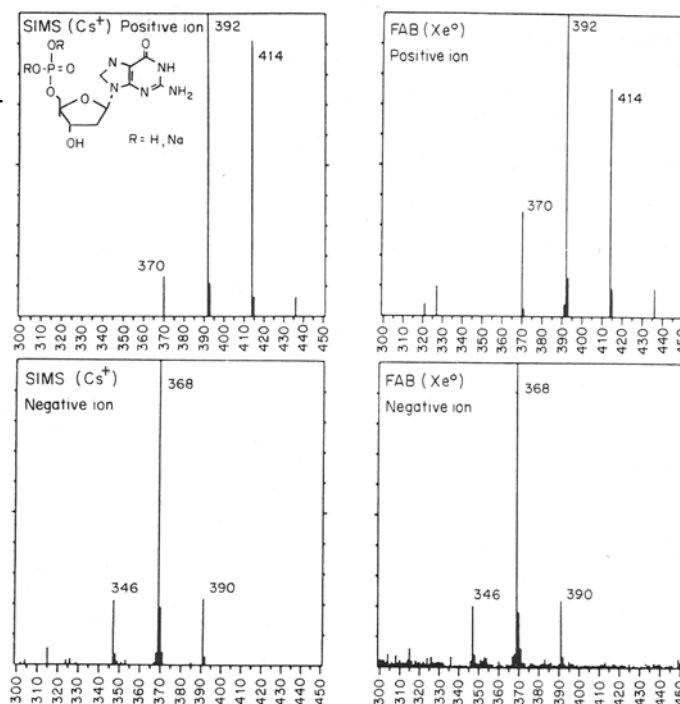
- Same as for FAB *except*
- target can get hotter than in FAB due to more energetic primary beam
- high-voltage arcs more common than FAB
- ion source usually requires more maintenance than FAB

Mass range

- *Moderate*: Typically 300 to 13,000 D.

Figure 4.5 shows a comparison of FAB and SIMS of 5'-deoxy-guanosine mono-phosphate $C_{10}H_{12}N_5O_7PR_2$.

Figure 4.5. Comparison of FAB and SIMS in + and – ion mode of 5'-deoxy-guanosine mono-phosphate $C_{10}H_{12}N_5O_7PR_2$. The molecular weight without R is 345, which the following values for the various cations: R= 1 H (-): 346; 1 Na (-): 368; 2Na/-1H (-): 390; 2H/1Na (+): 370; 2Na/1H (+): 392; 3Na (+): 414.



4.4. Laser Desorption. 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) or Fourier transform ion cyclotron resonance (FTICR) that is compatible with pulsed ionization methods. Magnetic sector mass spectrometers equipped with an array detector can also be used for the detection of ions produced by MALDI.

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)* 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.

Matrix-Assisted Laser Desorption Ionization (MALDI). The analyte is dissolved in a solution containing an excess of a matrix such as sinapinic acid (Fig. 4.6, bottom middle) 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. The matrix absorbs the energy from the laser pulse and produces a plasma that results in vaporization and ionization of the analyte (Figure 4.6, right).

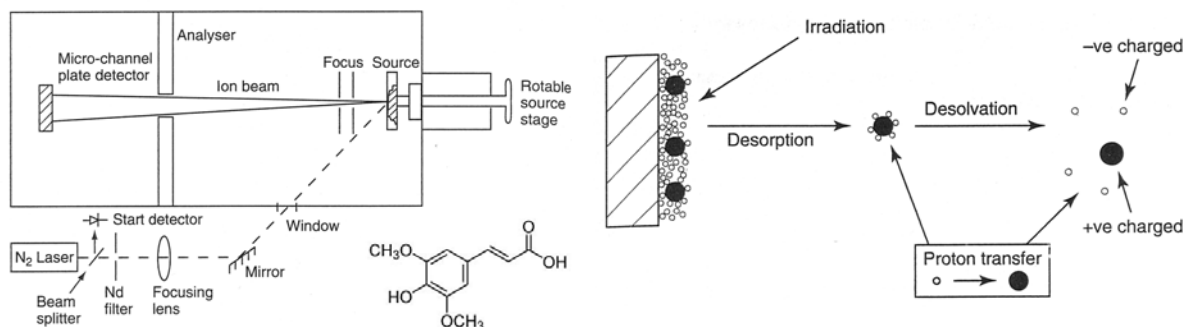


Figure 4.6. Desorption/Ionisation by MALDI (Barker). (left) instrument; (bottom middle) sinapinic acid, a matrix molecule; (right) irradiation, desorption, desolvation, and proton transfer.

Sample introduction

- direct insertion probe
- continuous-flow introduction

Benefits

- rapid and convenient molecular weight determination

Limitations

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

Mass range

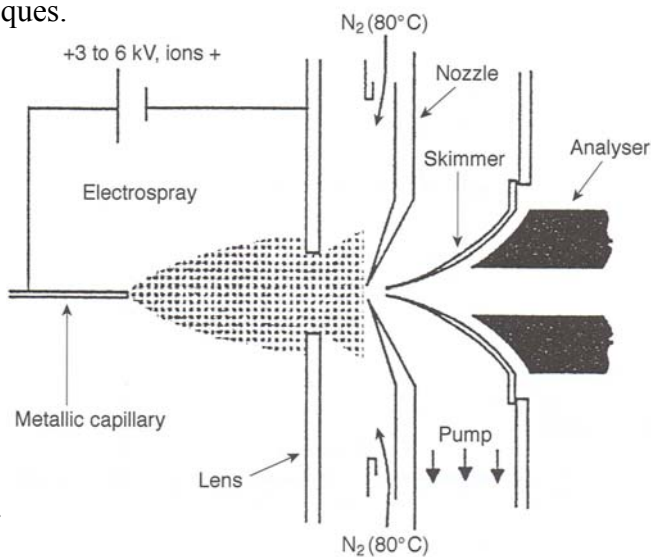
- *Very high* Typically less than 500,000 Da.

4.5. Atmospheric Pressure Ionization (Spray Methods). In these methods, a solution containing the analyte is sprayed, at atmospheric pressure, through an interface into the vacuum of the mass spectrometer ion source. A combination of thermal and pneumatic means is used to desolvate the ions as they enter the ion source. Solution flow rates can range from less than a microliter per minute to several milliliters per minute. These methods are well-suited for flow-injection and LC/MS techniques.

Electrospray Ionization (ESI). The sample solution is sprayed across a high potential difference (a few kilovolts) from a needle into an orifice in the interface (Figure 4.7).

Heat and gas flows are used to desolvate the ions existing in the sample solution.

Figure 4.7. Schematic representation of an electrospray source.



Electrospray ionization can produce multiply charged ions with the number of charges tending to increase as the

molecular weight increases. The number of charges on a given ionic species must be determined by methods such as:

- comparing two charge states that differ by one charge and solving simultaneous equations
- looking for species that have the same charge but different adduct masses
- examining the mass-to-charge ratios for resolved isotopic clusters

Sample introduction

- flow injection
- LC/MS
- typical flow rates are less than 1 microliter per minute up to about a milliliter per minute

Benefits

- good for charged, polar or basic compounds
- permits the detection of high-mass compounds at mass-to-charge ratios that are easily determined by most mass spectrometers (m/z typically less than 2000 to 3000).
- best method for analyzing multiply charged compounds
- very low chemical background leads to excellent detection limits
- can control presence or absence of fragmentation by controlling the interface lens potentials
- compatible with MS/MS methods

Limitations

- multiply charged species require interpretation and mathematical transformation (can sometimes be difficult)
- complementary to APCI. No good for uncharged, non-basic, low-polarity compounds (*e.g.* steroids)
- very sensitive to contaminants such as alkali metals or basic compounds
- relatively low ion currents
- relatively complex hardware compared to other ion sources

Mass range

- *Low-high* Typically less than 200,000 D.

Atmospheric Pressure Chemical Ionization (APCI). Similar interface to that used for ESI. In APCI, a corona discharge is used to ionize the analyte in the atmospheric pressure region. The gas-phase ionization in APCI is more effective than ESI for analyzing less-polar species. ESI and APCI are complementary methods.

Sample introduction

- same as for electrospray ionization

Benefits

- good for less-polar compounds
- excellent LC/MS interface
- compatible with MS/MS methods

Limitations

- complementary to ESI.

Mass range

- *Low-moderate* Typically less than 2000 D.

4.6. Hyphenated Techniques.

In GC-MS (Gas Chromatography-Mass Spectrometry), a gas chromatograph is connected to a mass spectrometer by a jet separator (Figure 4.8, top left) that selectively pumps off the (lighter) carrier gas (H_2 , He) molecules • and allows only the sample ○ molecules to enter the ionization chamber. In LC-MS (Liquid Chromatography-Mass Spectrometry) the connection is made as described in the previous section with thermospray or atmospheric pressure chemical ionization, i.e. ionization with remaining eluent as reaction gas.

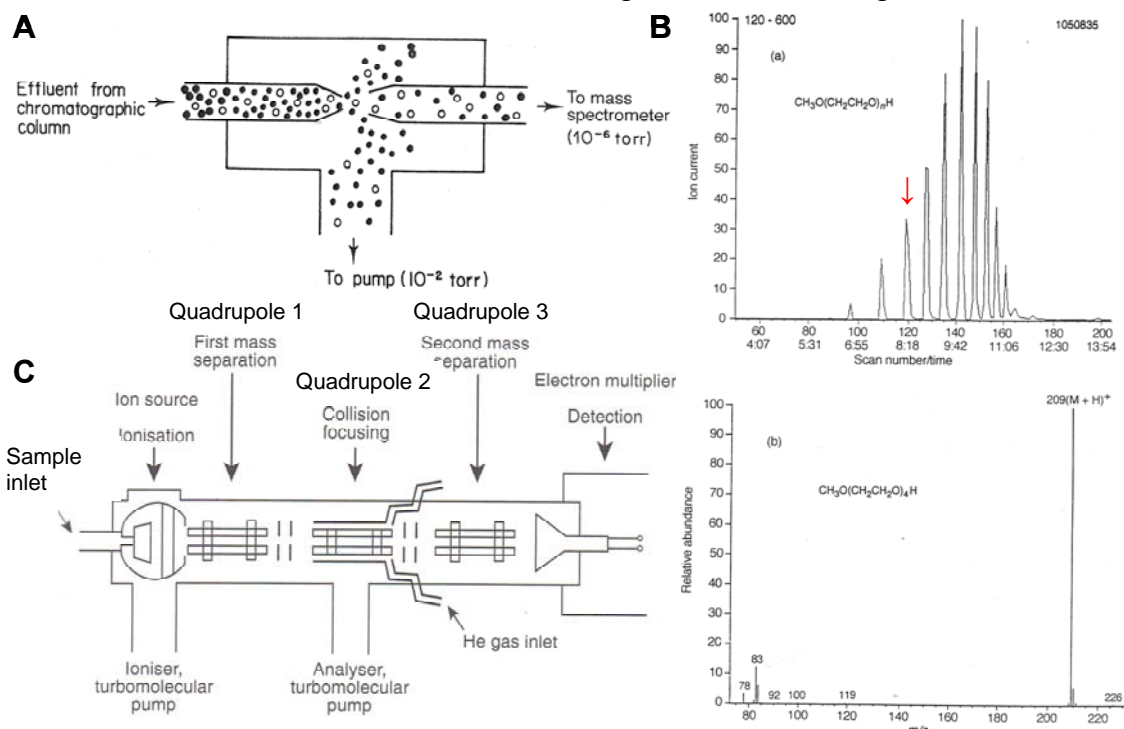


Figure 4.8. (top left) jet separator connection between GC and MS; (right) liquid chromatogram (top) and mass spectrum (bottom) of selected peak (red arrow ↓) of methoxy polyethylene glycol oligomers; (bottom left) tandem quadrupole mass spectrometer.

The example (Figure 4.8, right) shows a separation of methoxy polyethylene glycol oligomers by reversed phase HPLC, and MS analysis of the peaks with thermospray (jet with heating), MS taken at ↓. GC-MS and LC-MS are examples of 'hyphenated'-MS techniques. Tandem MS (also known as MS/MS, or MS^2) is a set-up with 3 quadrupole analysers (Figure 4.8, bottom left) which allows the fragmentation of selected ions to be studied by collision-induced dissociation (CID, or collision-induced fragmentation) in the central quadrupole element. The fragmentation of fragments can also be studied: MS/MS/MS or MS^3 , and again and again: MS^n .

Tandem MS can be run in a number of ways:

- i) Selection of an ion (m/z) with Q1, fragmentation in Q2, analysis in Q3 by a 'fragment-ion scan' or 'daughter scan' can answer fundamental regarding fragmentation mechanisms.
- ii) Analyse Q1 and select a fragment with Q3; a 'precursor-ion scan' or 'parent-ion scan': for classes of compounds that give a certain fragment
- iii) Set the difference between Q1 and Q3 to a certain value; a 'neutral loss scan': for classes of compounds that give a certain neutral loss.

4.7. Some commonly used chemicals in mass spectrometry

4.7.1. CI Reagent Gases

Methane:

- good for most organic compounds
- usually produces $[M+H]^+$, $[M+CH_3]^+$ depending on partial pressure $[M+C_2H_5]^+$ adducts
- adducts are not always abundant
- extensive fragmentation

Isobutane:

- usually produces $[M+H]^+$, $[M+C_4H_9]^+$ adducts and some fragmentation
- adducts are relatively more abundant than for methane CI
- not as universal as methane

Ammonia:

- fragmentation virtually absent
- polar compounds produce $[M+NH_4]^+$ adducts
- basic compounds produce $[M+H]^+$ adducts
- non-polar and non-basic compounds are not ionized

4.7.2. FAB matrices

Glycerol

- $C_3H_8O_3$, m/z 92.0473
- first and most widely-used FAB matrix.
- best choice for polar compounds

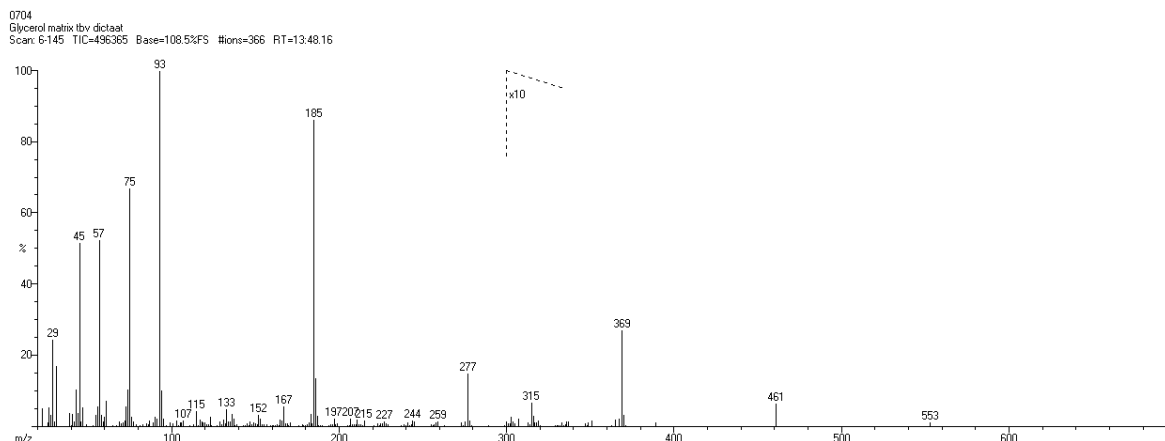


Figure 4.9. Positive ion-mode FAB spectrum of Glycerol.

1-thioglycerol

- $C_3H_8O_2S$, m/z 108.0245
- similar uses to glycerol, but may enhance $M+1$ abundance

- more volatile than glycerol, evaporates quickly

0706
thio-glycerol matrix tbv dictaat
Scan: 1-13 TIC=143978 Base=37.9%FS #Ions=102 RT=1:07.769

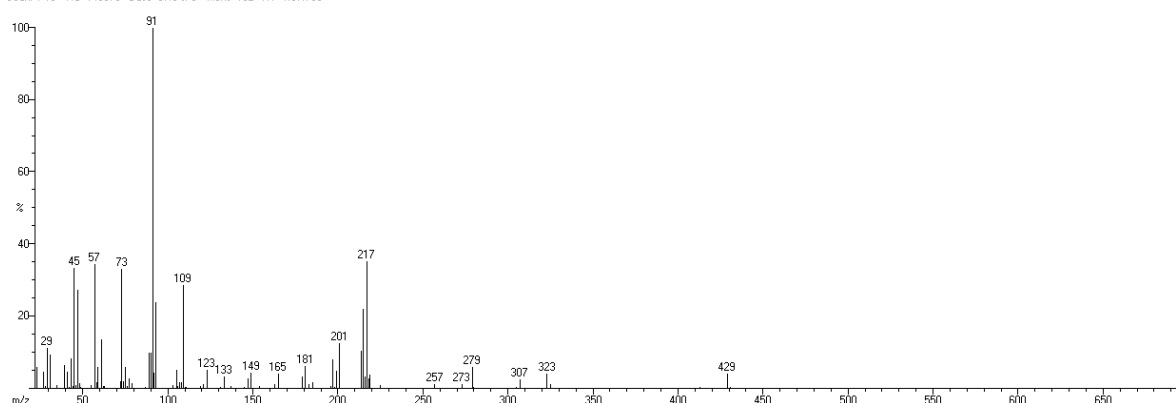


Figure 4.10. Positive ion-mode FAB spectrum of thioglycerol.

3-nitrobenzyl alcohol (NBA)

- $C_7H_7NO_3$, m/z 153.0416
- best choice for less polar compounds (*e.g.* chlorophyll) and many organometallics
- can produce radical molecular ions as well as protonated or cationized molecular ions

0702
NBA ter controle
Scan: 18-112 TIC=828939 Base=84%FS #Ions=1002 RT=1:15.65

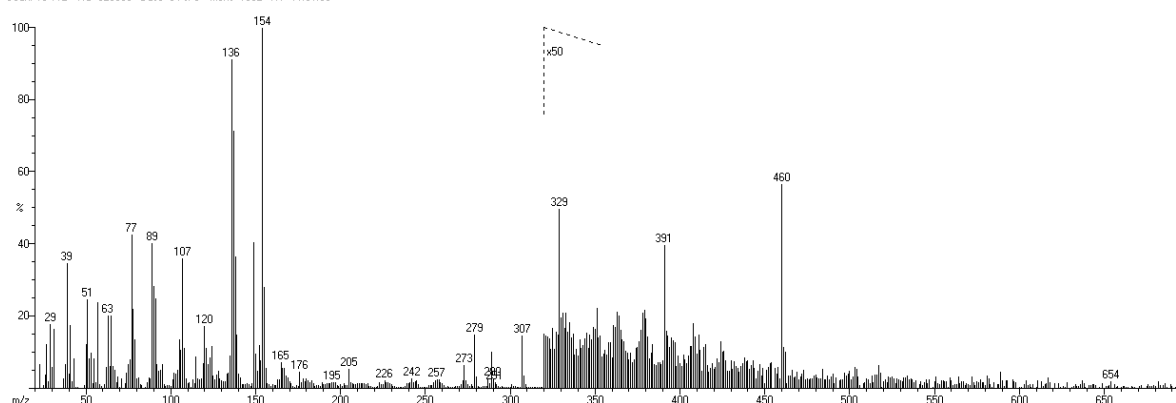


Figure 4.11. Positive ion-mode FAB spectrum of NBA.

2-nitrophenyl octyl ether (NPOE)

- $C_{14}H_{21}NO_3$, m/z 251.1521
- only FAB matrix with no reactive hydrogen
- good choice for reactive compounds such as organometallics

0703
NPOE tbv dictaat
Scan: 1-277 TIC=1044505 Base=97%FS #Ions=465 RT=2:33.10

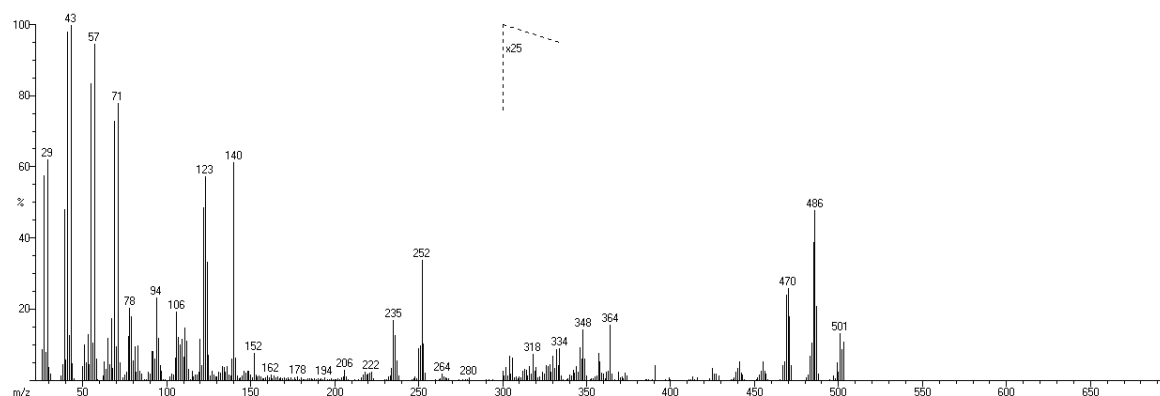


Figure 4.12. Positive ion-mode FAB spectrum of nitro-phenyl-octyl-ether

Triethanolamine

- $\text{C}_6\text{H}_{15}\text{NO}_3$, m/z 149.1052
- good matrix for negative-ion FAB
- enhances $[\text{M}-\text{H}]^-$ formation.

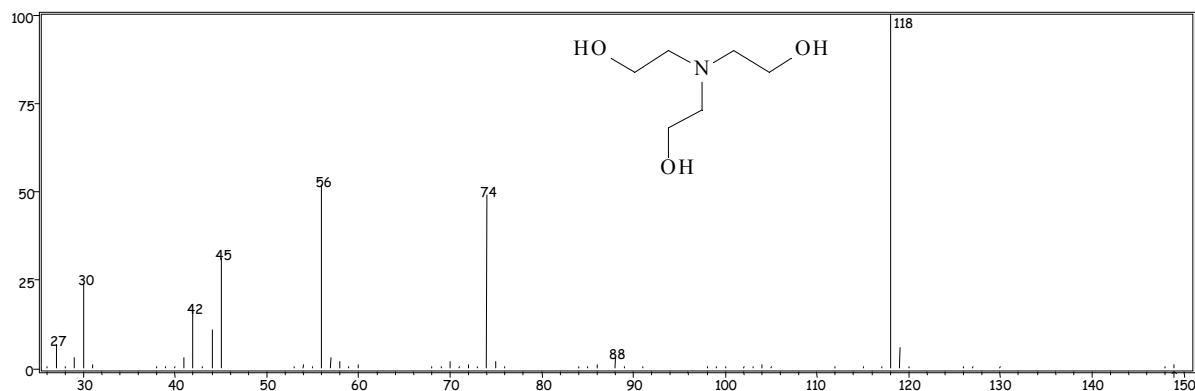


Figure 4.13 EI mass spectrum of triethanolamine.

Diethyl phthalate (DEP)

- *bis*(2-ethylhexyl) phthalate
- $\text{C}_{24}\text{H}_{38}\text{O}_4$, m/z 390.54
- Similar in use to NPOE
- Widespread contaminant in solvents, gives characteristic 149 peak in EI mass spectra
- Use care to avoid contaminating the mass spectrometer

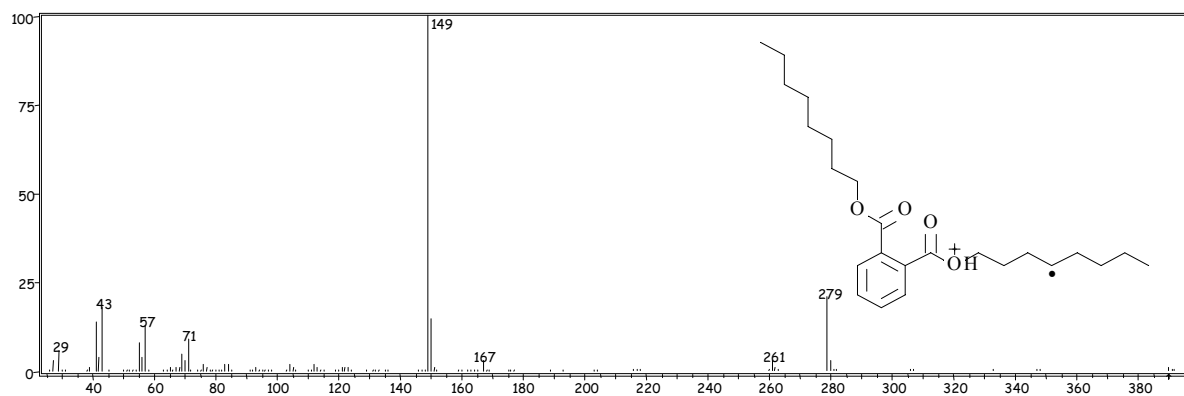


Figure 4.14. EI mass spectrum of di-octyl phthalate.

5. Mass Spectrometry of Biomolecules

5.1. Introduction: In the general lecture notes on Mass Spectrometry general principles of ionization, separation, detection, and fragmentation are discussed for organic molecules. This appendix deals specifically with biomolecules such as proteins and peptides, oligo- and polynucleotides, and oligo- and polysaccharides. The common principles will be discussed with peptides/proteins as examples.

5.2. Ionization methods: Because of the high molecular weights of biomolecules, it is not possible to bring them in the vapour phase so that electron impact or chemical ionization can be applied. Therefore ionization techniques such as fast-atom bombardment (FAB) and the related secondary ion mass spectrometry (SIMS), electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI) are needed. The typical ranges of application of these ionization techniques are listed in Table 5.1.

Table 5.1. Mass spectrometry of biomacromolecules.

| Ionization method | Detection limit (pmol) | Common application range (M_r) ^a | Precision (%) | Analyser |
|----------------------------------|------------------------|---|---------------|------------------------|
| Fast-atom bombardment | 1-50 | 6000 | 0.05 | Magnetic or quadrupole |
| Electrospray (also with TOF) | 0.01-0.10 | < 130,000 | 0.01 | Magnetic or quadrupole |
| Matrix-assisted laser desorption | 0.001-0.01 | < 300,000 | 0.05 | Time-of-flight |

^a) (M_r)^a, molecular weight relative to that of ^{12}C ($\equiv 12$), often given in the non-S.-I. unit Da (Dalton)

Because of the large molecular weights and the large number of atoms in the formula, it is important to be aware of the effects of isotopes that contribute to the satellite peak pattern. For an (A+1) element like C, for which the natural abundance of the minor isotope, ^{13}C , is approx. 1 %, it can be expected that if the nominal mass (calculated on the basis of the most abundant isotopes) is M, the satellite peak at M+1 has higher intensity if there are 100 or more C in the formula. Similar considerations apply to the other (A+1) and (A+2) elements in biomolecules; the resulting patterns can be calculated. As an example, the FAB-MS of the isotopic cluster of insulin is shown in Figure 5.1 at various resolutions.

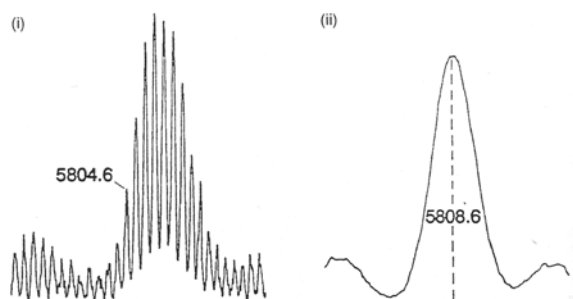


Figure 5.1B. FAB-MS of the isotopic cluster of insulin, $\text{C}_{257}\text{H}_{383}\text{N}_{65}\text{O}_{77}\text{S}_6$, $M_r = 5801$, at a resolution of (i, left) 6000, and (ii, right) 500.

5.2.1. Electrospray Ionization: Because FAB techniques cannot be applied to molecules larger than 6,000, electrospray ionization (Fig. 5.2) is the method applied to most biomolecules. A solution of the molecule is squeezed through a capillary and subjected to an

electrostatic potential, which results in a static charge (excess H^+ or Na^+ ions) on the droplets, and exposed to a stream of cold nitrogen gas followed by vacuum, by which the solvent molecules are selectively evaporated/removed.

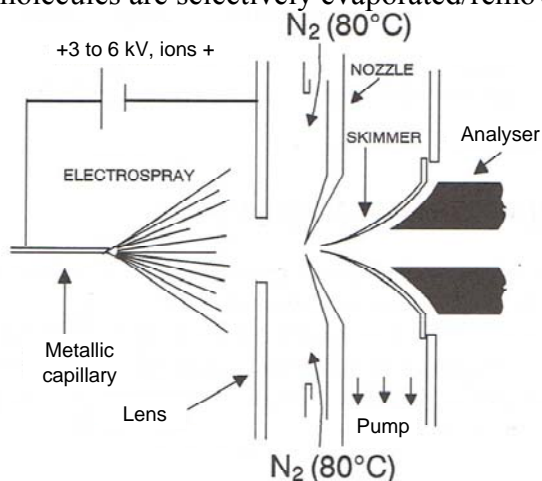


Figure 5.2dHCS. Electrospray source. (J. A. Loo et al., Anal. Biochem. 179 (1989) 404)

As a result of the electrospray process, the molecule is charged by one or multiple H^+ and/or Na^+ ions. This results in the appearance of charge clusters, i.e. m/z peaks which represent the same molecule, but with different charges; the pattern can be deconvoluted to the M_r which corresponds to the m/z peak with a single charge. The example of the deconvolution of the pattern of m/z peaks of the protein equine apomyoglobin to the M_r values of approx. 17,000 is shown in Fig. 5.3.

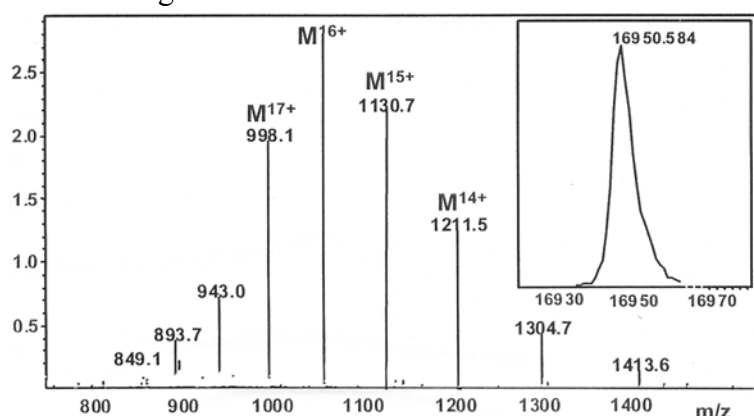


Figure 5.3. ESI of equine apomyoglobin.

In a native protein, fewer basic residues become exposed and charged in the ElectroSpray Ionisation process than in the corresponding denatured (unfolded) protein (Fig. 5.4).

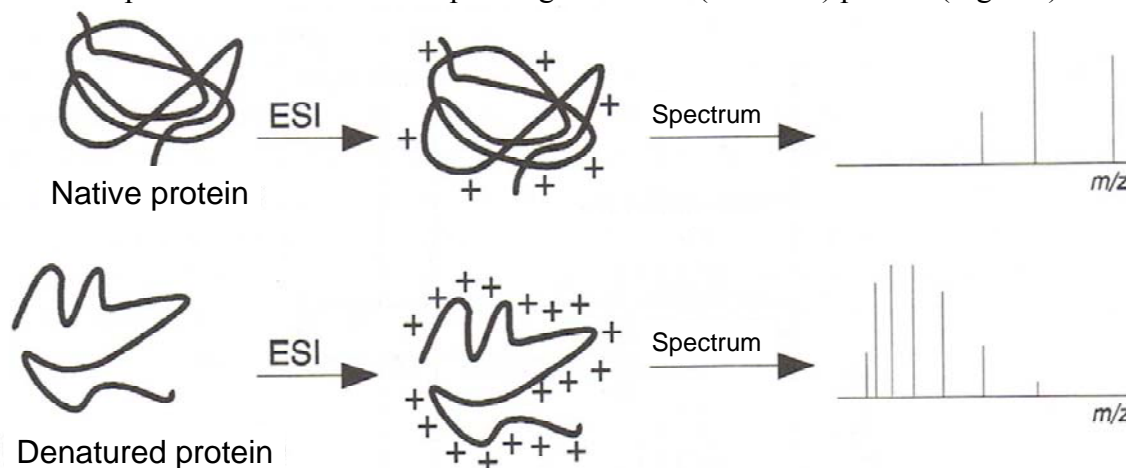
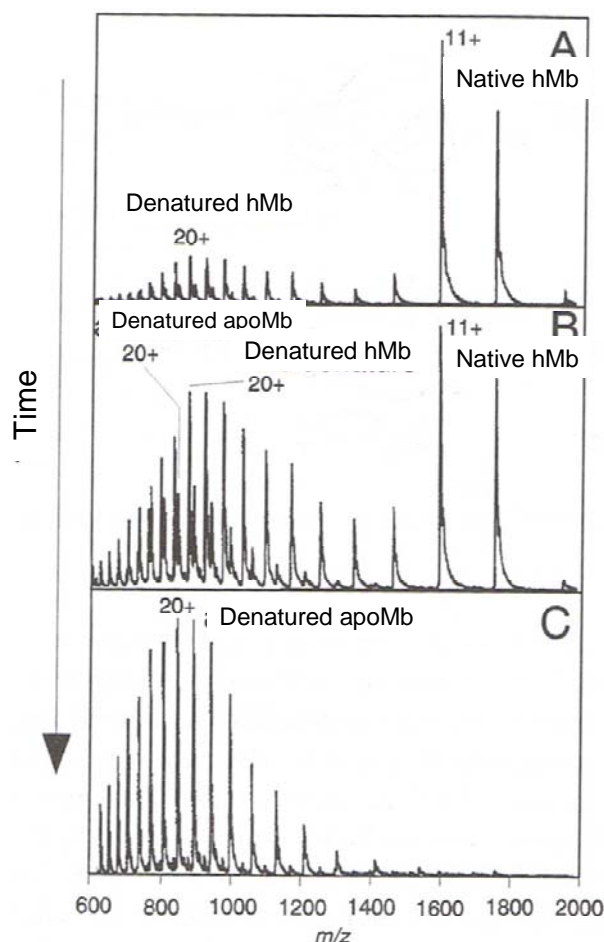


Figure 5.4. Distribution of charged states characteristic of native and denatured proteins.

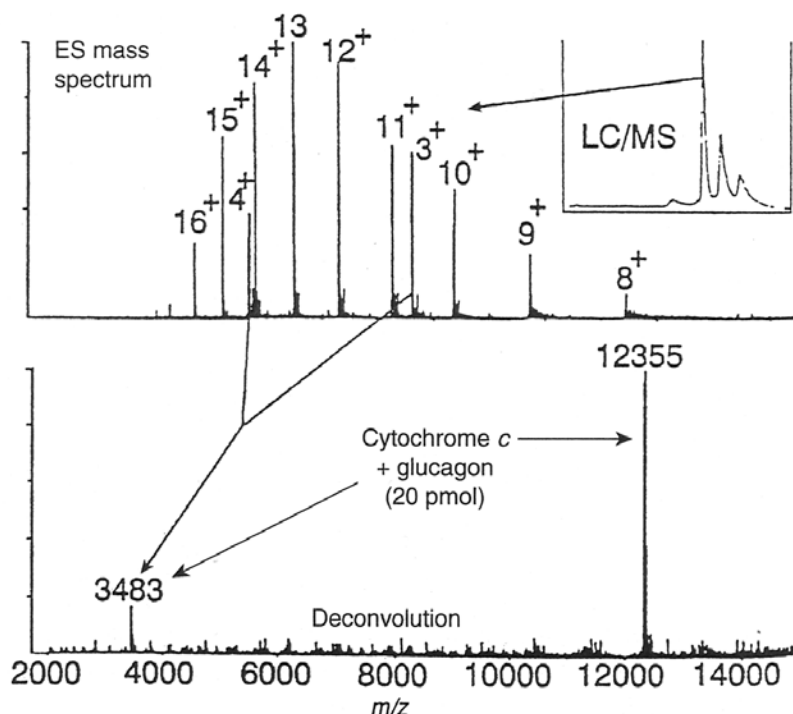
The process of denaturation of a protein in an acidic environment can be followed in time. The example of myoglobin, where denaturation also results in loss of the heme cofactor, is given in Fig. 5.5. In Fig. 5.5A, the sequence starts with native myoglobin (hMb) with a small amount of denatured myoglobin; in Fig. 5.5B, the amount of denatured myoglobin has increased at the expense of native protein, and apomyoglobin (which has lost the heme cofactor) appears; in Fig. 5.5C, the myoglobin is completely unfolded, and has lost all the heme.

Figure 5.5dHCS. Results of the denaturation of myoglobin in an acidic environment followed in time. Three species are detected: native hMb (native myoglobin with heme), denatured hMb (denatured myoglobin with heme) and denatured aMb (denatured apomyoglobin). L. Konerman et al. Biochemistry 34 (1997) 6448.



Mixtures of biomolecules can give interfering patterns of charge clusters, which can be deconvoluted to the correct molecular weights. Figure 5.6 shows the example of the mixture of the electron transport protein Cytochrome c, and Glucagon, a peptide hormone ($C_{153}H_{225}N_{43}O_{49}S$) from the *insulae Langerhantis* in the pancreas, with activity opposite to insulin

Figure 5.6 (Finnigan MAT documentation). Deconvolution of the ESI of a LC-MS fraction shows that it contains cytochrome-c and glucagon.



5.3. Peptides and proteins: As can be seen in the overview in Figure 5.7, the 20 natural amino acids all have different weights, except for the isomers (same element composition) Leu and Ile, which have exactly the same mass (isomers), and Lys and Gln (different element composition) which have the same nominal mass (isobars).

| | | | | | |
|-------|--|---|--|--|--|
| G 57 | Ala 71.03712 | Arg 156.10112 | Asn 114.04293 | Asp 115.02695 | Cys 103.00919 |
| A 71 | $\text{H}_2\text{N}-\text{CH}(\text{CH}_3)-\text{COOH}$ | $\text{H}_2\text{N}-\text{CH}(\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2)-\text{COOH}$ | $\text{H}_2\text{N}-\text{CH}(\text{CH}_2\text{C(=O)NH}_2)-\text{COOH}$ | $\text{H}_2\text{N}-\text{CH}(\text{CH}_2\text{C(=O)OH})-\text{COOH}$ | $\text{H}_2\text{N}-\text{CH}(\text{CH}_2\text{SH})-\text{COOH}$ |
| S 87 | | | | | |
| P 97 | | | | | |
| V 99 | Glu 129.04260 | | Gln 128.131 | His 137.05891 | Ile 113.08407 |
| T 101 | $\text{H}_2\text{N}-\text{CH}(\text{CH}_2\text{CH}_2\text{C(=O)OH})-\text{COOH}$ | $\text{H}_2\text{N}-\text{CH}(\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2)-\text{COOH}$ | $\text{H}_2\text{N}-\text{CH}(\text{CH}_2\text{CH}_2\text{C(=O)NH}_2)-\text{COOH}$ | $\text{H}_2\text{N}-\text{CH}(\text{CH}_2\text{CH}_2\text{CH}_2\text{NH})-\text{COOH}$ | $\text{H}_2\text{N}-\text{CH}(\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_3)-\text{COOH}$ |
| C 103 | | Gly 57.02147 | | | |
| I 113 | | $\text{H}_2\text{N}-\text{CH}(\text{H})-\text{COOH}$ | | | Pro 97.05277 |
| L 113 | | | | | |
| N 114 | | Lys 128.09497 | Met 131.04049 | Phe 147.06842 | |
| D 115 | | $\text{H}_2\text{N}-\text{CH}(\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2)-\text{COOH}$ | $\text{H}_2\text{N}-\text{CH}(\text{CH}_2\text{CH}_2\text{SCH}_3)-\text{COOH}$ | $\text{H}_2\text{N}-\text{CH}(\text{CH}_2\text{C}_6\text{H}_5)-\text{COOH}$ | $\text{H}_2\text{N}-\text{CH}(\text{CH}_2\text{C}_6\text{H}_4\text{NH})-\text{COOH}$ |
| Q 128 | Leu 113.08407 | | | | |
| K 128 | $\text{H}_2\text{N}-\text{CH}(\text{CH}_2\text{CH}(\text{CH}_3)\text{CH}_3)-\text{COOH}$ | | | | |
| E 129 | | | | | |
| M 131 | | | | | |
| H 137 | | | | | |
| F 147 | | | | | |
| R 156 | Trp 186.07932 | Ser 87.03203 | Thr 101.04768 | Val 99.06842 | |
| Y 163 | $\text{H}_2\text{N}-\text{CH}(\text{CH}_2\text{C}_8\text{H}_6\text{NH})-\text{COOH}$ | $\text{H}_2\text{N}-\text{CH}(\text{CH}_2\text{OH})-\text{COOH}$ | $\text{H}_2\text{N}-\text{CH}(\text{CH}(\text{OH})\text{CH}_3)-\text{COOH}$ | $\text{H}_2\text{N}-\text{CH}(\text{CH}(\text{CH}_3)_2)-\text{COOH}$ | $\text{H}_2\text{N}-\text{CH}(\text{CH}_2\text{C}_6\text{H}_4\text{OH})-\text{COOH}$ |
| W 186 | | | | | |

Figure 5.7. Molecular weights (-18, H₂O) of the amino acids: left: single letter code with nominal mass; right: structure with accurate mass.

Fragmentation of positive ions of peptides and proteins, once created by FAB, ESI, or MALDI, can occur spontaneously and be detected by separating the ions. It can also be induced by so-called tandem mass spectrometry in which the ions are made to undergo collisions (collision-induced dissociation) with inert gas molecules (e.g. Ar) at low pressure in an ion trap or quadrupole. Peptides and proteins have a convenient tendency to fragment somewhere near the amide bond. The fragment peaks arising from fragmentation due to breaking of the C α -C, C-N, or N-C α bonds are labelled by the so-called Roepstorff-Fohlman-Biemann nomenclature, of which details are given in Fig. 5.8.

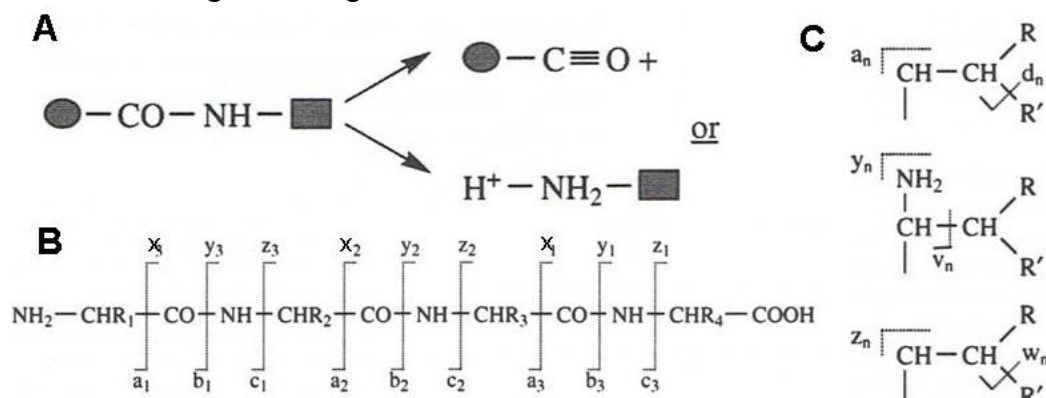


Fig. 5.8. Fragmentation of protonated peptide in FAB/MS. (A) Positive ions may be generated at the C- or N-terminal side of peptide bonds. (B) Main-chain fragmentation. (C) Side-chain fragmentation. Note that d_n and w_n-type ions allow distinction of isomers such as Leu and Ile.

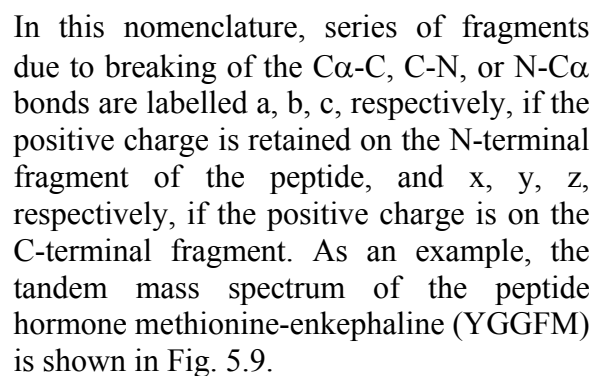


Figure 5.9dHCS. Fragmentation spectra at high and low energy of methionine-enkephalin (YGGFM) labelled with Roepstorff-Fohlman-Biemann nomenclature (M. F. Bean et al., Anal. Chem. 63 (1991) 1473)

$$\left[\begin{array}{c} \text{R} \\ | \\ \text{H}_2\text{N}-\text{CH}-\text{CO}-(\text{NH}-\text{CH}-\text{CO})_n-\text{NH}-\text{CH}-\text{COOH} + \text{H} \\ | \quad | \quad | \\ \text{R} \quad \text{R} \quad \text{R} \\ \text{a}_n \quad \text{b}_n \quad \text{c}_n \\ \text{x}_n \quad \text{y}_n \quad \text{z}_n \end{array} \right]^+$$

$$\begin{array}{l} \text{H}-(\text{HN}-\text{CH}-\text{CO})_{n-1}^+-\text{NH}=\text{CH}^+ \\ \text{a}_n \end{array} + \begin{array}{l} \text{R}_n \quad \text{R} \\ | \quad | \\ \text{CO}-\text{HN}-\text{CH}-\text{CO}-(\text{NH}-\text{CH}-\text{CO})_{n-1}-\text{OH} \\ \text{x}_n \end{array}$$

$$\begin{array}{l} \text{H}-(\text{HN}-\text{CH}-\text{CO})_{n-1}^+-\text{NH}-\text{CH}^+-\text{C}=\text{O} \\ \text{b}_n \end{array} + \begin{array}{l} \text{R}_n \quad \text{R} \\ | \quad | \\ \text{H}_3\text{N}-\text{CH}-\text{CO}-(\text{NH}-\text{CH}-\text{CO})_{n-1}-\text{OH} \\ \text{y}_n \end{array}$$

$$\begin{array}{l} \text{H}-(\text{HN}-\text{CH}-\text{CO})_{n-1}^+-\text{NH}-\text{CH}-\text{CO}-\text{NH}_3^+ \\ \text{c}_n \end{array} + \begin{array}{l} \text{R}_n \quad \text{R} \\ | \quad | \\ \text{CH}-\text{CO}-(\text{NH}-\text{CH}-\text{CO})_{n-1}-\text{OH} \\ \text{z}_n \end{array}$$

Fig. 5.10dHCS. Principal fragmentation paths for peptides in tandem mass spectrometry/collision induced dissociation.

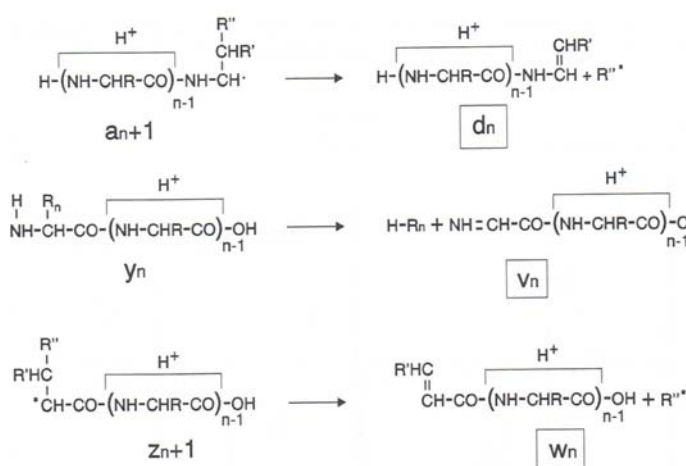


Fig. 5.11dHCS. Fragmentation paths that yield ions characteristic for the amino acid side chains.

The possible side chain fragmentations that can follow main chain fragmentation are given in more detail in Figure 5.11. Fragments of type a_n and z_n can undergo fragmentation between $C\beta$ and $C\gamma$ in the side chain next to the site where the main chain fragmentation has occurred; this leads to the series of fragments d_n and w_n in which the positive charge is retained on the N- and C-terminal side of the site of main chain fragmentation, respectively. This allows discrimination between Ile and Leu side chains. This type of fragmentation does not occur for His, Phe, Tyr, and Trp; they undergo fragmentation of the y_n to yield v_n fragments by loss of the complete side chain instead.

As shown in Fig. 5.12, two kinds of fragments can result from a double cleavage of the peptide backbone. i) The so-called 'internal fragment' is obtained by loss of both the N- and C-termini, which is usually just a nuisance, but relatively strong for Pro: this residue is unique because the secondary amine is a relatively strong base. ii) The iminium ions confirm the amino acid composition, but give no sequence information.

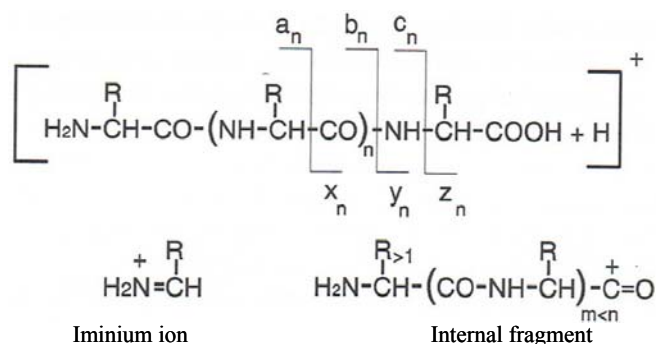


Fig. 5.12dHCS. Fragments resulting from double cleavage of the peptide backbone.

If a complete series of fragments is present, the sequence of a peptide from the N- to the C-terminus can be established from the b-type ions; the complimentary reverse information can be obtained from the series of y-type ions. In the example in Fig. 5.13, the mass difference between b_2 and b_3 is 97, which points to the presence Pro in the 3rd position from the N-terminus; the difference between y_1 and y_2 is 147, which points to the presence of Phe in the 2nd position from the C-terminal side. In the example, Ile and Leu are also discriminated; the m/z values for w_3 , w_4 , and w_5 , indicate the presence of Leu in corresponding positions, whereas that for w_8 points to Ile. PT, PTL, PTLT are internal fragments involving Pro, and P, F, X are iminium ions due to Pro, Phe, and Ile/Leu.

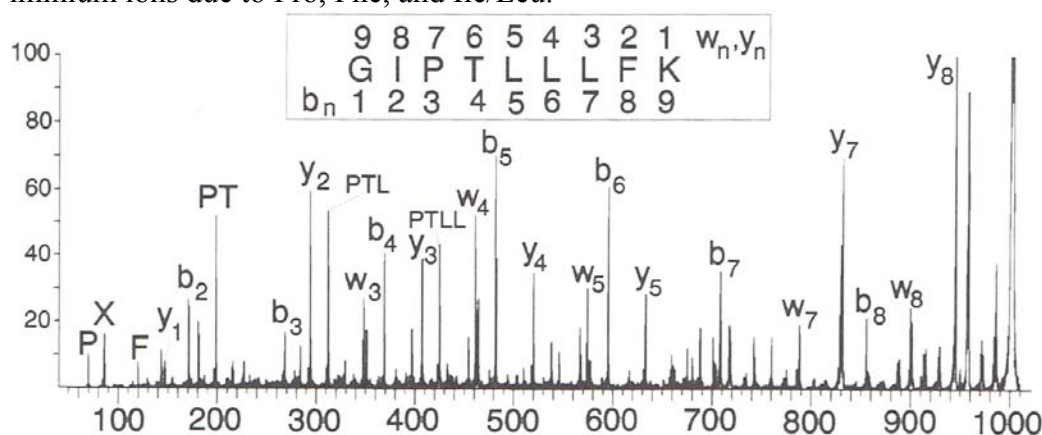


Fig. 5.13dHCS. FAB/MS/MS spectrum of a peptide with mass 1,001 and sequence Gly-Ile-Pro-Thr-Leu-Leu-Phe-Lys taken at high energy (K. Biemann, Biomed. Environ. Mass Spectrom. 16 (1988) 99)

5.3.2. Post-translational modifications. Paim-I, a 73-amino acid peptide inhibitor of α -amylase can be digested by *S. aureus* V8 protease and the degradation products analysed by FAB-MS before and after separation by HPLC (Fig. 5.14). In the mixture (Fig. 5.14 top left) the fragments with m/z ($=m$) 2,523 and 3,066 do not correspond to any peptide in the sequence. They could be accounted for by the presence of S-S bridges (see Fig. 5.14 bottom for structure), e.g. between Cys 42 and 70, as the mass of 3,066 corresponds approximately to the sum of those of the peptides Gly 37-Glu55 (1,698) + His 66-Ser 73 (1,370). Indeed the m/z peaks at 1,698 and 1,370 are also observed in the mixture, as the disulfide bridges are easily reduced during FAB-MS analysis. This is confirmed by analysis of the purified peptide with m/z = 2,836; this is proposed to contain the disulfide-linked peptides Ala 1-Glu 14 (1,366) + Ser 15-Asp 26 (1,472) which are both present (Fig. 5.14, top right).

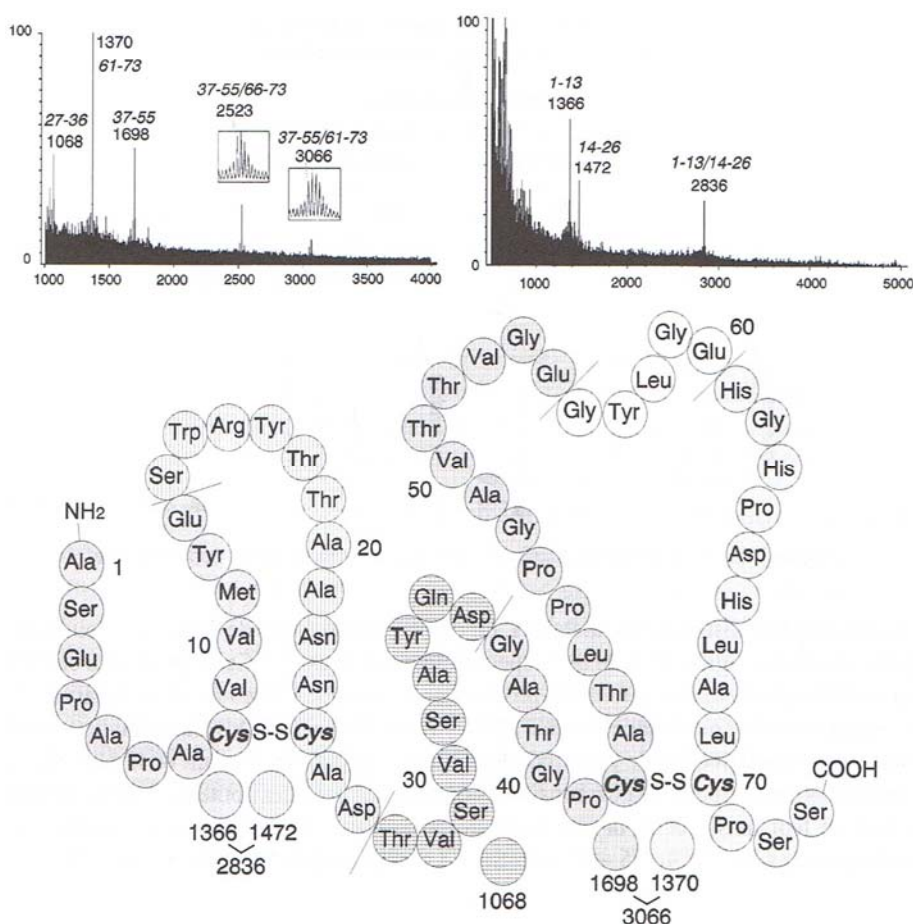


Fig. 5.14dHCS. FAB spectra of a digest (protease V8) of the amylase inhibitor Paim I, and of a peptide recovered from the mixture and purified by HPLC (S. Akashi et al., Biomed. Environ. Mass Spectrom. 15 (1988) 541)

The peptide sequence of bradykinine contains 2 possible phosphorylation targets, viz. Tyr-5 and Ser-6. The tandem mass spectra obtained by FAB MS/MS (FAB followed by CID) allow the localization of the phosphate on the tyrosine in position 5.

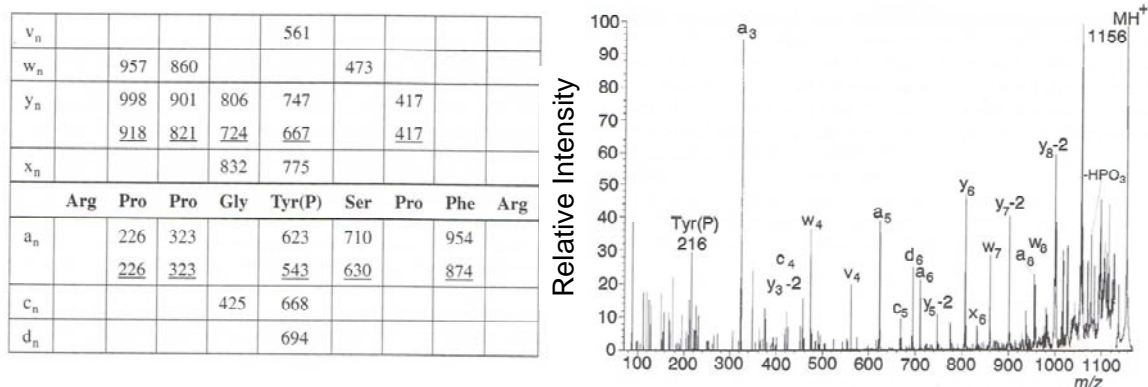


Fig. 5.15dHCS. FAB/MS/MS spectrum of phosphorylated bradykinine [Tyr₅ (P)]. The underlined masses correspond to fragments without phosphate (B. W. Gibson & P. Cohen, Methods in Enzymology, 193 (1990) 501).

Mass spectrometric analysis also provides a valuable check on the purity of synthetic peptides such as the example SNYTFSQM shown in Fig. 5.16. The minor peaks are attributed to oxidation of Met ($M + 16$), a peptide retaining a *t*Bu protective group ($M + 56$), and a O-trifluoroacetyl group on Ser or Thr ($M + 96$; identified as Ser-1 by tandem MS). The species with m/z 849 is a deletion peptide lacking Gln ($M - 128$).

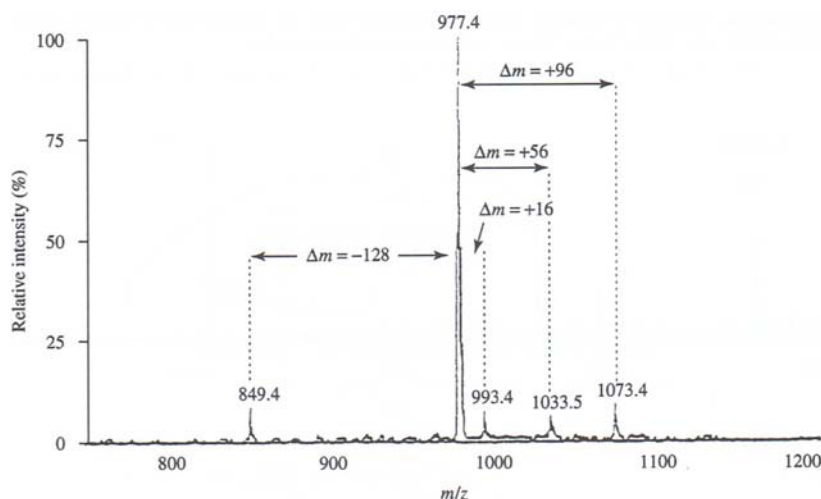


Fig. 5.16Sh. ESI/MS (triple quadrupole) of a peptide synthesized by Merrifield (Fmoc) method with sequence Ser-Asn-Tyr-Thr-Phe-Ser-Gln-Met (M_r 977). (Metzger et al., Anal. Biochem. 219 (1994) 261).

Mass spectrometric analysis allows both mutations and post-translational modifications and their positions to be established. As discussed above, side-chain fragmentations have to be analysed in order to be able to distinguish Lys/Gln or Ile/Leu. Post-translational modifications detected by MS include:

- N-terminus: Acylation, N-formylation, N-formyl methionine, pyroglutamic acid, aminopeptidases
- Internal: Glycosylation, phosphorylation, deamidation, desulphide formation, oxidation, endopeptidases and endoproteinases
- C-terminus: Carboxypeptidase, ragged ends

The pattern of peptide fragments can be simplified by converting the N-terminus to a negative charge by sulphonation (Fig. 5.17); the more reactive ϵ -amino groups of Lys side chains should be modified first by e.g. guanidinylation. Incubation with the trypsin protease, which is selective for cleavage only on the C-terminal side of Lys and Arg (but not on the homo-Arg which results from Lys-guanidinylation), leads to the production of peptides with only cationic residues on the C-terminal side, and only negative charge on the N-terminal side if this is sulphonated. In a combination with electrophoresis, the Lys residues of a protein in an electrophoresis gel are first guanidinylated, then digested by trypsin, and subsequently sulphonated and subjected to PSD-MALDI (post-source decay matrix-assisted laser desorption). Only a series of positively charged y-ions is observed, which allows sequence to be determined.

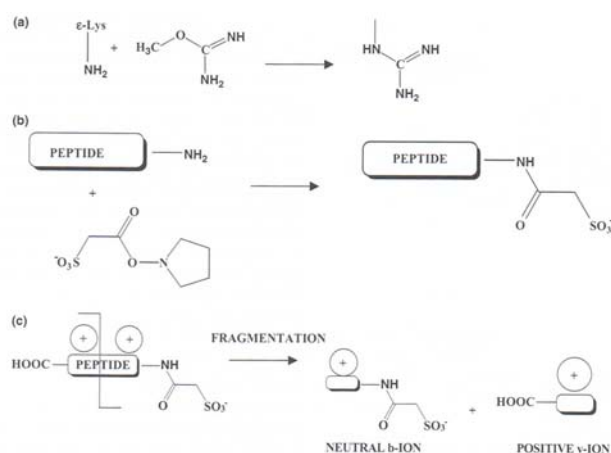


Fig. 5.17Sh. Terminal Sulphonation. a) Tryptic digests produce C-terminal lysines and arginines. Lysine can be converted to homoarginine by guanidinylation. (b) N-terminal amino groups react with an N-hydroxysuccinimide ester of 3-sulphopropionic acid. (c) C-terminus has positive charge due guanidinylation. Fragmentation by post-source decay MALDI produces a y-ion from each peptide which is detected by MS and an uncharged b-ion which is not. Mass differences between adjacent y-ions allow calculation of the sequence.

In proteomics, protein mixtures are analysed in high throughput. In the surface-enhanced laser desorption ionization time-of flight mass spectrometry (SELDI-TOF MS, Fig. 5.18) approach, chromatography is combined with MALDI-TOF. The chips a, b, c, d have derivatized surfaces with distinct chromatographic properties, viz. ion exchange, reverse phase, affinity (including immobilize metal ions), and hydrophobic chromatography, which allows fractionation of the proteosome into subproteosomes. The results are presented as if obtained by electrophoresis.

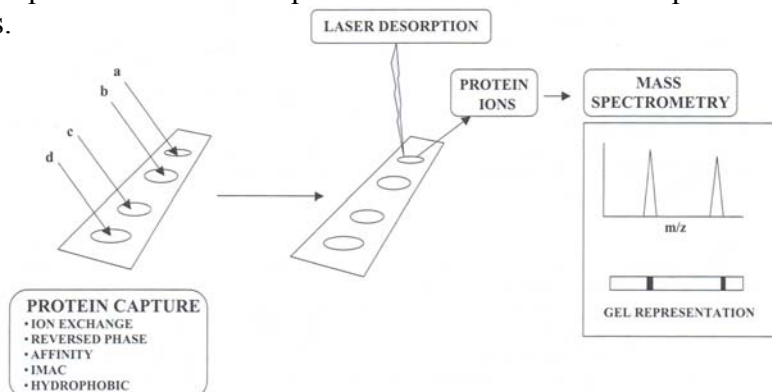
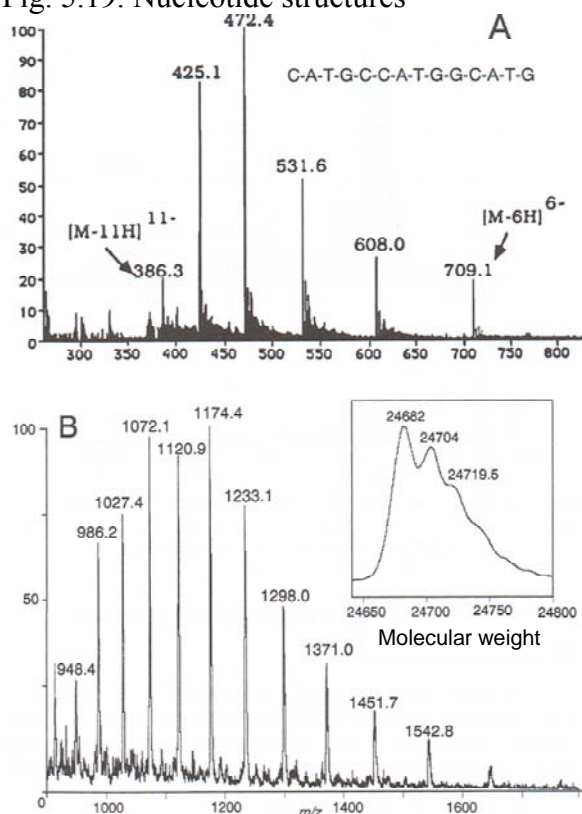


Fig. 5.18. SELDI-MS. This is distinguished from MALDI MS by the use of protein chips in a range of formats based on chromatographic interaction. The output of the experiment is presented as an MS spectrum as if the separation was performed in an electrophoresis gel.

5.4. Polynucleotides: The structures of DNA and RNA and their bases (DNA: Adenine, Guanine, Cytosine, Thymine; RNA: Uracil instead of Thymine) are given in Fig. 5.19.

Fig. 5.19. Nucleotide structures

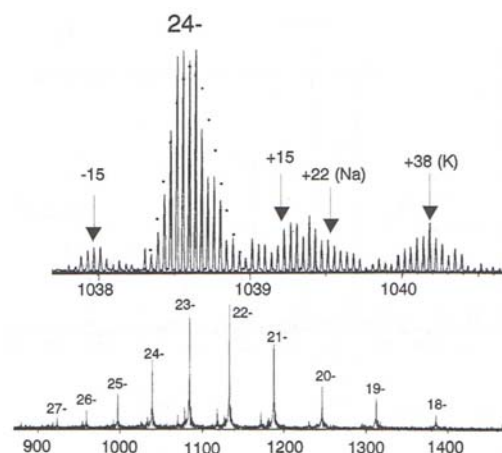


Negative ion ESI is the method of choice for the analysis of whole polynucleotides (Fig. 20). The phosphate groups determine the negative charge, and charge clusters of negative ions are observed. There are often various degrees of association with the H^+ , Na^+ , and/or K^+ counterions.

Fig. 5.20dHCS. A) Negative ion ESI spectrum of a synthetic oligonucleotide of M_r 4260. B) ESI spectrum of *E. coli* tRNA^{Val} with a calculated M_r of 24,681. (P. Limbach et al., J. Am. Soc. Mass Spectrom. 6 (1995) 27.

This is illustrated in more detail in Fig. 5.21, which shows the satellite peak pattern of the ESI FT-MS of the Phe tRNA of *E. coli*. Note that a difference in nominal mass of 1 results in a peak separation in m/z of approx. 0.04, because in m/z , $z = 24$ for the 24⁻ peak of the charge cluster. In addition of the peaks resulting from the substitution of remaining H⁺ for Na⁺ or K⁺ (peaks with a higher nominal mass of respectively 22 and 38), there are also weak contributions of (de)methylated material.

Fig. 5.21dHCS. FT-MS ESI spectrum of charge clusters of tRNA^{Phe} (bottom) with an expansion of the region of the satellite peaks of the 24⁻ anion (top). (D. P. Little et al. Proc. Natl. Acad. Sci. USA 92 (1995) 2318).



The polynucleotides have a tendency to give main-chain fragmentation around the phosphate diester. The nomenclature is analogous to that of fragmentation of proteins around amide bonds, except that there are 4 bonds that can be broken (Fig. 5.22, left): the C-O, O-P, P-O, and O-C bonds, leading to the respective a, b, c, d and w, x, y, and z series, depending on whether the negative charge is on the 5' or the 3' end of the nucleotide. It should be noted that, despite the intended analogy, the d and w fragments, which refer to side chain fragmentations in the case of proteins, have a different meaning in the case of the polynucleotides. The FAB MS of the oligo-nucleotide UGUU (Fig. 5.22, right) shows additional peaks due to loss of H₂O (-18) or addition of HPO₃ (+80).

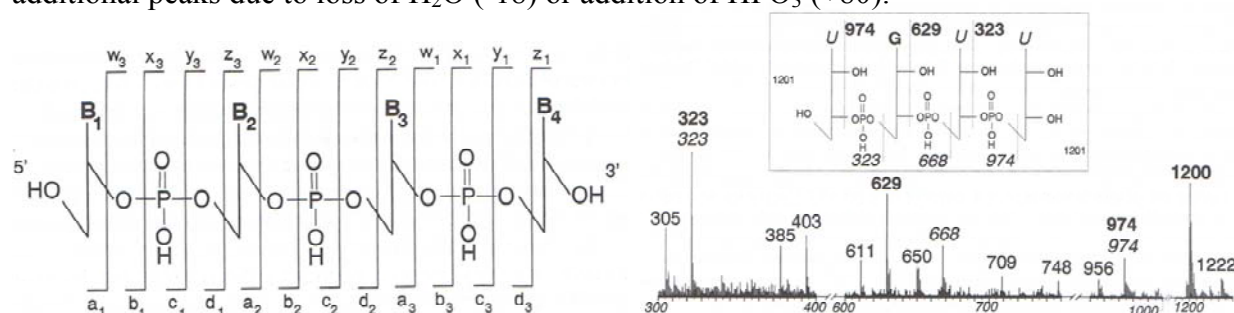


Fig. 5.22dHCS. (left) Nomenclature of the dissociation products of oligonucleotides; (right) Negative ion FAB spectrum of an oligonucleotide with sequence UGUU (L. Grotjahn, Mass Spectrometry in Biomedical Research, Ed. S. J. Gaskell, Wiley, New York, 1986)

The negative charges act as bases and induce specific fragmentation pathways (Fig. 5.23)

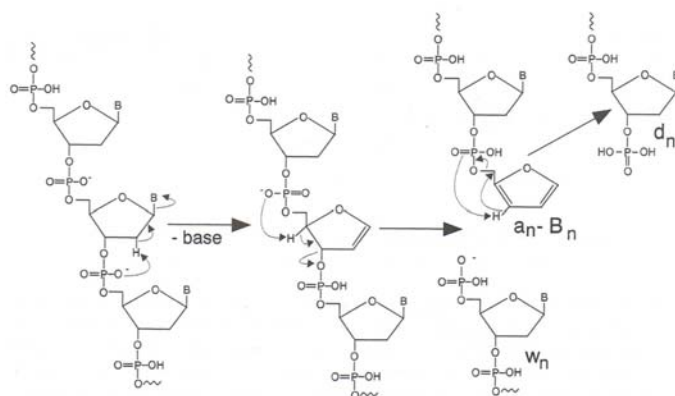


Fig. 5.23dHCS. Fragmentation paths of oligonucleotides for which a charge close to the site of cleavage is necessary.

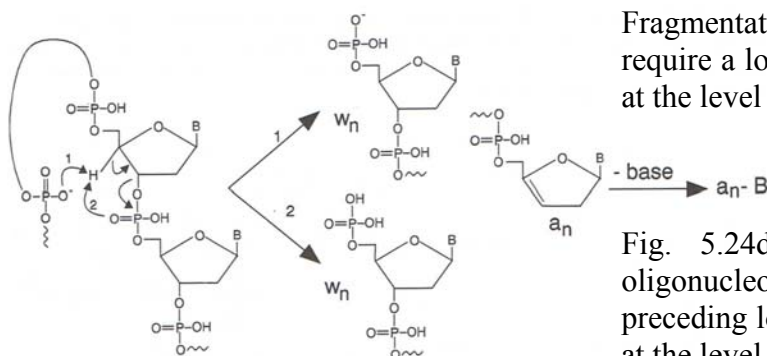


Fig. 5.24dHCS. Fragmentation paths of oligonucleotides that do not require either the preceding loss of the nucleotide, nor a charge at the level of the site of cleavage.

5.5. Polysaccharides: Because of the various possibilities of linking the building blocks and their similarity in molecular weight, mass spectrometry of polysaccharides is even more complex than that of proteins and polynucleotides: an interesting area for specialists. While it is generally not possible to distinguish the anomeric configuration of the glycosidic bonds nor the diastereoisomers of the building blocks, it should be possible to determine the sequence, branching motifs, and regioisomers of the glycosidic bonds.

The various possibilities of fragmenting around a glycosidic bond are shown in Fig. 5.25, top left. The spectrum of the peracetylated linear polysaccharide LNF-I (Fig. 5.25, left middle) shows a series of oxonium ions B: B₁ (273) Fuc-Ac₃, B₂ (561) Fuc-Hex-Ac₆, B₃ (848) Fuc-Hex-GlcNAc-Ac₈, B₄ (1136) Fuc-Hex-GlcNAc-Hex-Ac₁₁, and B₅ (1424) Fuc-Hex-GlcNAc-Hex-Ac₁₄, which allow the sequence to be determined, albeit without distinction between the diastereoisomeric hexoses (Hex = Glc or Gal).

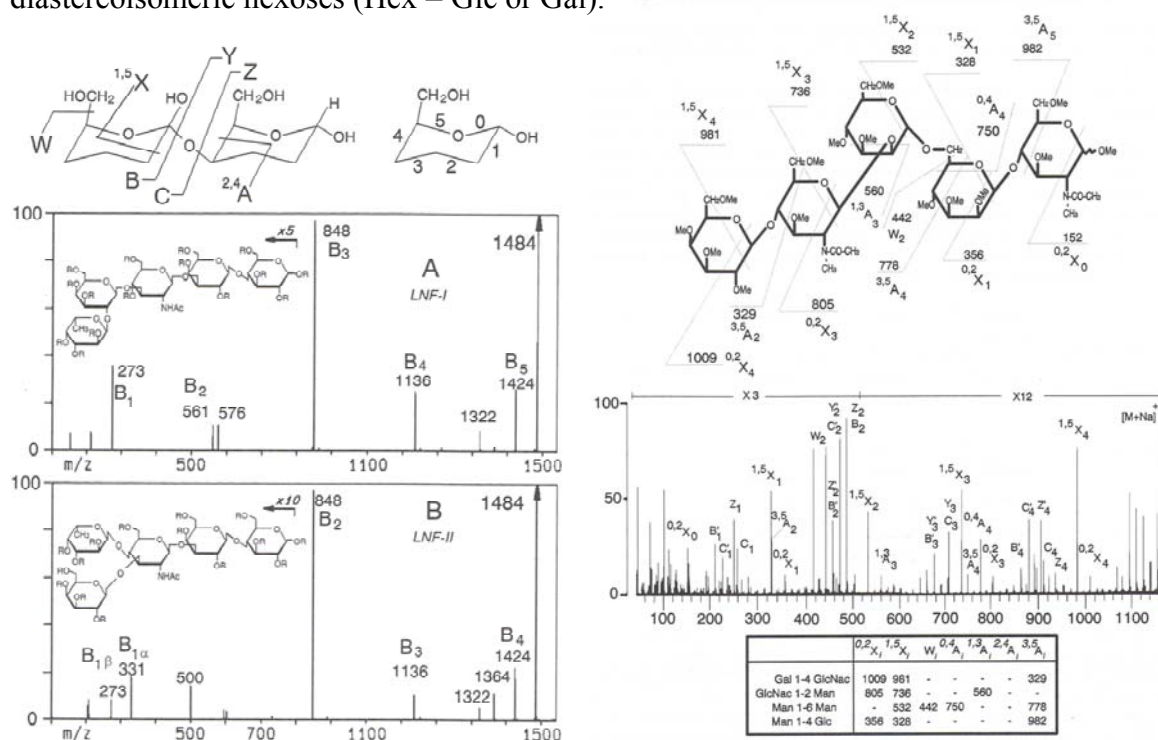


Fig. 5.25dHCS. Left top) nomenclature proposed by Domon and Costello, middle and bottom: FAB MS/MS CID spectra of 2 peracetylated oligosaccharide branched isomers of lacto-N-fucopentose (Fuc₁Gal₂Glc₁GlcNAc₁) LNF-I and LNF-II. (B. Domon et al., Biomed. Environ. Mass Spectrom. 19 (1990) 390). Right) FAB MS/MS CID of (M + Na)⁺ of an oligonucleotide at high energy, allowing the determination of the glycosidic linkage isomers. (J. Lemoine et al., J. Am. Soc. Mass Spectrom. 4 (1997) 197).

In contrast, the spectrum of the branched isomer LNF-II (Fig. 5.25, left bottom) shows two monosaccharide ions $B_1\beta$ (273) Fuc-Ac₃ and $B_1\alpha$ (331) Hex-Ac₄ and no disaccharide ion B_2 in addition to the ions B_{3-5} with the same m/z as for the linear isomer; this allows the position of the branching to be established.

The fragmentation of a carbohydrate ring in which the hydroxyl groups at C1 and C4 are involved in glycosidic bonds to the other carbohydrates can only lead to the formation of the fragments of type $^{1,5}X_i$, $^{0,2}X_i$, $^{3,5}A_i$, and $^{2,4}A_i$ (Table 5.2; Fig. 5.25, top right); in case of glycosidic bonds involving the hydroxyl groups at C1 and C2, only $^{1,5}X_i$ and $^{1,3}A_i$ can be formed. The FAB MS/MS CID of the Na^+ -adduct of a methylated oligosaccharide (spectrum and Table, Fig. 5.25 right, middle and bottom) shows how the fragmentation information can be used to determine the pattern of glycosidic linkages.

Table 5.2dHCS.

Potential diagnostic peaks for the determination of the position of the glycosidic bonds.

| Positions of -OH groups in the ring involved in glycosidic bonds | Fragments | | | | | | |
|--|-----------|-------------|-------------|-------------|-------------|-------------|-------------|
| | W_i | $^{0,2}X_i$ | $^{1,5}X_i$ | $^{0,4}A_i$ | $^{1,3}A_i$ | $^{2,4}A_i$ | $^{3,5}A_i$ |
| 1,2 | - | - | + | - | + | - | - |
| 1,3 | - | + | + | - | + | + | - |
| 1,4 | - | + | + | - | - | + | + |
| 1,6 | + | + | + | + | - | - | + |

5.6. Overview: We have found that peptides and proteins can be analysed with FAB, ESI, or MALDI as the ionization techniques. ESI gives charge clusters which can be deconvoluted to the M_r . Denaturation exposes more residues that can be charged, and can lead to loss of non-covalently bound cofactors. Sequence information can be obtained by fragmentation, which can be induced by collision with inert atoms. Amino acids with identical weight can be distinguished by side chain fragmentations. Mutations and post-translational modification are easily identified.

In polynucleotides the charge is dominated by the phosphates and negative ion mass spectrometry is the method of choice. Partial exchange of H^+ for Na^+ and K^+ ions is commonly observed.

For polysaccharides information can usually be obtained on the sequence, the branching pattern, and the positions of the glycosidic linkages, but not on the diastereoisomers of the building blocks, nor the anomeric configuration of the glycosidic bonds.

6. Literature, Sources

- J. Barker, Mass Spectrometry, Analytical Chemistry by Open Learning, Wiley, 2nd Edition (1999).
- N. B. Cech & C.G. Enke, "Practical Implications of Some Recent Studies in Electrospray Ionization Fundamentals", Mass Spectrometry Reviews, 2001, 20, 362-387.
- M. Guilhaus, Journal of Mass Spectrometry 30 (1995) 1519-32. 'Principles and Instrumentation in Time-of-Flight Mass Spectrometry'
- A. G. Harrison, "Chemical Ionisation Mass Spectrometry", CRC Press Inc., Florida (1983).
- D. Henneberg und K. Casper, Z. Anal. Chem., 227, 2412 (1967).
- M. Hesse, H. Meier, B. Zeeh, Spektroskopische Methoden in der organischen Chemie, Thieme, Stuttgart/New York, 6th edition, 2002 (also available in English)
- E. de Hoffmann, J. Charette, V. Stroobant, Spectrométrie de masse. Course et exercices corrigés. Dunod, Paris (1999) (also available in English)
- I. Howe, D. H. Williams & R. D. Bowen, "Mass Spectrometry Principles and Applications", McGraw-Hill Inc., New York (1981).
- M. Karplus and R. N. Porter, "Atoms & Molecules".
- Lecture notes of Bruker mass spectrometry course, F. Laukien
- N. M. M. Nibbering & R.H. Fokkens, "Mass Spectrometry, Course on Modern Spectroscopy", University of Twente Netherlands.
- N. M. M. Nibbering, "College Massaspectrometrie", Amsterdam
- N. M. M. Nibbering, "Organische Massaspectrometrie: reaktiemechanismen van ontledingen van ionen in de gasfase", Amsterdam
- F. W. McLafferty, "Interpretation of Mass Spectra", University Science Books, Mill Valley, California (1980);
F. W. McLafferty F. Tureček, "Interpretation of Mass Spectra", University Science Books, Mill Valley, California (1993)
- R. I. Reed, "Mass Spectrometry", Academic Press, London (1964)
- J. D. Roberts and M. C. Caserio, "Basic Principles of Organic Chemistry", W.A. Benjamin Inc., NY 1965.
- U. P. Schlunegger, "Advanced Mass Spectrometry", Pergamon Press, Oxford (1983).
- D. Sheehan, Physical Biochemistry, Principles and Applications (2nd edition), Wiley-Blackwell, 2009
- D.A. Skoog & D.M. West, "Principles of Instrumental Analysis", Holt , Rhinehart and Winston Inc. NY 1971;
D.A. Skoog & J. J. Leary, "Principles of Instrumental Analysis", 4th Ed., Saunders College Publishing.
- Steven Weinberg, "Bouwstenen van het atoom", Wetenschappelijke Bibliotheek (Natuur & Techniek), 1986.
- Dudley H. Williams & Ian Fleming, "Spektroskopische methoden zur Strukturaufklaerung", Georg Thieme Verlag Stuttgart, 1979 p162-216