containing one less hydroxyl group at *m/z* 491 and one more at *m/z* 523. These last ions are completely buried in the noise in the source FAB spectrum.

4.3 Collision-Activated Decomposition or Collision-Induced Dissociation

Tandem mass spectrometry requires the fragmentation of precursor ions selected by the first analyser in order to allow the second analyser to analyse the product ions. According to the Warhaftig diagram (see Chapter 7), the ions leaving the source can be classified into three categories. The first category of ions, with a lifetime greater than 10^{-6} s, reaches the detector before any fragmentation has occurred. The second category, with a lifetime smaller than 10^{-7} s, fragments before leaving the source and only the fragments are detected. The last category, called metastable ions, have an intermediate lifetime. These ions are stable enough to be selected by the first analyser while containing enough excess energy to allow their fragmentation before they reach the second analyser. The probability associated with this phenomenon is relatively low (1 %) because their number is small and they spend a very short time in the reaction region. If precursor ions undergo a collisional activation (CA), that is an increase in their internal energy that induces their decomposition, then the situation described above is much improved. This collision-activated decomposition (CAD) technique, also known as collision-induced dissociation (CID), allows one to increase the number of precursor ions that fragment in the reaction region and also the number of fragmentation pathways. The structural analysis thus becomes easier.

An overall view shows the CID process as a sequence of two steps. The first step is very fast (10^{-14} to 10^{-16} s) and corresponds to the collision between the ion and the target when a fraction of the ion translational energy is converted into internal energy, bringing the ion into an excited state. The second step is the unimolecular decomposition of the activated ion. The collision yield then depends on the activated precursor ion decomposition probability according to the theory of quasi-equilibrium or RRKM. This theory is explained elsewhere. Let us recall that it is based on four suppositions:

1. The ion dissociation time is long with respect to the formation time and the excitation time.
2. The dissociation rate is low with respect to the rate of the redistribution of the excitation energy among all of the ion internal modes.
3. The ion achieves an internal equilibrium condition where the energy is distributed with an equal probability among all of the internal modes. Considering that an ion with *N* non-linear atoms has $3N - 6$ vibration modes, it is easy to understand how the collision yield decreases in a manner inversely proportional to the ion mass.
4. The dissociation products that are observed result from a series of competitive and consecutive reactions.

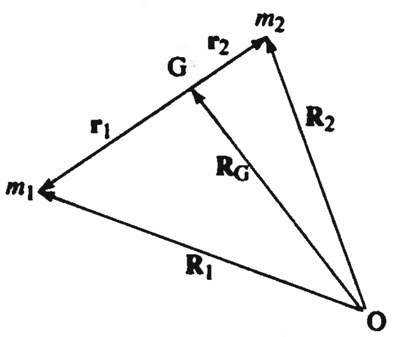
As a consequence of these suppositions, CID is an ‘ergodic’ ion activation method, which allows a redistribution of the energy in the vibrational modes of the ion because the dissociation rate is slower than the rate of energy randomization. In these conditions, the fragmentation pathways depend on the amount of energy deposited and not on the method of ion activation used. As the energy is distributed with an equal probability among all of the internal modes of the ion, this leads preferentially to cleavage sites at the weakest bonds. For the same reason, molecules with more atoms will need more energy, or more time, to dissociate. Modern ionization techniques allow the production of ions with high-molecular-weight compounds. This is why other ion activation methods that are being developed now increase the energy transfer [8].

Several methods exist that activate the ions through collisions. The most common one consists of colliding the low- or high-energy accelerated ions with gas molecules as immobile targets. To achieve collisional activation in MS/MS instruments with spatially separate analysers, a collision cell is placed between the two mass analysers. This cell often corresponds simply to a small chamber with entrance and egress apertures and contains an inert target gas at a pressure sufficient for collisions with ions to occur. In MS/MS instruments based on time-separated mass analysis steps, an inert gas is simply introduced into the ICR or the ion trap. In some elaborations of these experiments, it is introduced only into a certain region or at a particular time in the operating sequence using a pulsed valve. An article describing the history of this technique [9] and the fundamental aspects are detailed in the literature [10–12].

4.3.1 Collision Energy Conversion to Internal Energy

The kinetic energy for internal energy transfers is governed by the laws concerning collisions of a mobile species (the ion) and a static target (the collision gas).

Consider an ion of mass m_1 hitting a target of mass m_2. If $\mathbf{R}$ represents the position vectors in the laboratory reference frame O, and $\mathbf{r}$ represents the position vectors in the reference frame linked to the centre of gravity G, we see on the following diagram that



$$\mathbf{R}_1 = \mathbf{R}_G + \mathbf{r}_1 \quad \text{and} \quad \mathbf{R}_2 = \mathbf{R}_G + \mathbf{r}_2$$

Defining the centre of gravity as $(m_1 + m_2)\mathbf{R}_G = m_1\mathbf{R}_1 + m_2\mathbf{R}_2$ then

$$\mathbf{r}_1 = m_2(\mathbf{R}_1 - \mathbf{R}_2)/(m_1 + m_2) \quad \text{and} \quad \mathbf{r}_2 = -\mathrm{m}_1(\mathbf{R}_1 - \mathbf{R}_2)/(m_1 + m_2)$$

Differentiating with respect to t,

$$\mathbf{u}_1 = m_2\mathbf{g}/(m_1 + m_2) \quad \text{and} \quad \mathbf{u}_2 = -\mathrm{m}_1\mathbf{g}/(m_1 + m_2)$$

with

$$\mathbf{g} = \mathbf{v}_1 - \mathbf{v}_2 \quad \text{and} \quad m_1\mathbf{u}_1 + m_2\mathbf{u}_2 = 0$$

According to the law of conservation of momentum, the kinetic energy of a rapid particle colliding with a static target ($\mathbf{v}_2 = 0$) cannot be entirely converted into internal energy. The kinetic energy available for conversion, $\mathbf{E}_t$, termed 'relative kinetic energy', is actually the kinetic energy in the centre of mass reference frame. Indeed, in this G frame the momentum is equal to zero. We thus obtain

$$E_t = (m_1u_1^2 + m_2u_2^2)/2 = \mu g^2/2$$

with

$$\mu = m_1m_2/(m_1 + m_2)$$

Thus

$$E_t = E_km_2/(m_1 + m_2) \quad \text{where} \quad E_k = m_1v_1^2/2$$

The kinetic energy is conserved in an *elastic* collision

$$\mu g^2/2 = \mu g'^2/2$$

In an *inelastic* collision, a part Q of the kinetic energy, at a maximum equal to E_t, is converted into internal energy:

$$\mu g^2/2 = Q + \mu g'^2/2$$

Thus, the conservation of energy and of momentum imply that only a fraction of the translational energy is converted into internal energy under inelastic conditions. This energy fraction is given by the following equation:

$$E_{\mathrm{cm}} = E_{\mathrm{lab}}\frac{M_t}{M_i + M_t}$$

where M_i is the ion mass; M_t the target gas mass; E_{lab} the ion kinetic energy in the laboratory frame of reference; and E_{cm} the maximum energy fraction converted into internal energy. Consequently, an increase in the ion kinetic energy or in the target gas mass increases the energy available for the conversion. This energy decreases as a function of $1/M_i$.

For instance, a 100 u ion with a kinetic energy of 10 eV colliding with argon (atomic mass 40) has a maximum increase in its internal energy amounting to

$$10\frac{40}{40 + 100} = 2.86\,\mathrm{eV}$$

At low collision energy this maximum will be almost reached, but at high collision energy, that is in the range of kiloelectronvolts, a fraction of this maximum will be converted to internal energy. Always keep in mind that 1 eV per ion is equal to around 100 kJ mol^{-1}.

transition. The energy thus acquired is then redistributed in the form of vibrational energy. A bond cleavage may result.

Helium is the most common target gas used in CID studies at high energy. In fact, it minimizes the dissociation reaction, that is the neutralization of the precursor ion and the deviation of the product ions beyond the angle allowed for the focalization of ions into the second sector of the spectrometer. However, helium is not very efficient in transferring internal energy, so using a heavier gas such as argon or xenon allows the collision yield to be increased.

The internal energy that is acquired during the ions' stay in the collision cell is on average 1–3 eV with an energy dispersion that can reach 15 eV [18, 19]. Only a small fraction of the energy available is actually converted. Let us recall that the maximum energy that can be converted in a system where an ion with mass 1000 Da has an energy of 4 keV and where the collision gas is helium is 16 eV. In addition, only collisions with an angular deviation of less than 1° are observable through this technique. Mechanisms producing a great angular dispersion of the products are thus rarely observed.

4.3.3 Low-Energy Collision (Between 1 and 100 eV)

Low-energy CID spectra are measured using triple quadrupole, ion trap, ICR or hybrid instruments. For tandem mass spectrometers in space, the collision chamber is most often a quadrupole in the RF mode only, which allows one to focus the ions that are angularly dispersed by the collision. The pressure difference between the collision cell and the rest of the analyser is obtained through differential pumping.

At low energy, the ions' excitation energy is mostly of a vibrational nature [20] because the interaction time between an ion with mass 200 Da at an energy of 30 eV with a target of a few angstroms is about 10^{-14} s, corresponding approximately to the bonds' vibration period.

The nature of the collision gas is more important than it is for the high-energy collisions. Normally, heavier gases such as argon, xenon or krypton are preferred because they allow the transfer of more energy.

The energy that is deposited is slightly lower than at high energy but has a weaker dispersion. However, the collision yields are extremely high when compared with the energy available. This is due on the one hand to the great focusing characteristic of RF-only quadrupoles and on the other to the length of the collision chamber that allows multiple collisions. Let us recall that the maximum energy that can be converted in such a system where an ion with m/z 1000 has a kinetic energy of 20 eV and where the collision gas is xenon is 2.3 eV.

4.4 Other Methods of Ion Activation

One of the main inconveniences with CID is the limitation of the energy transferred to an ion and thus the limitation of its degree of fragmentation. MS/MS is used more and more to fragment large molecules. When the ions are large, the energy is distributed on a greater number of bonds. The result is a slower reaction rate of the fragmentation. Furthermore, a collision gas must be introduced into the instrument, compromising the vacuum. In order to avoid these inconveniences, other ion activation methods were developed [21].

Another activation method, which is performed without gas, uses collisions with a solid surface [22, 23]. This method is called surface-induced dissociation (SID). In practice,