CHAPTER 3

The Mass Spectrometer

3.1 BASIC COMPONENTS

All mass spectrometers consist of three basic components, the:

(i) *ion source*,
(ii) *mass analyser*,
(iii) *ion detector*.

The role of the *ion source* is to introduce molecules into the mass spectrometer and convert them to a charged or ionised form. The ion source like the rest of the mass spectrometer is usually, though not always, held at a low pressure. Mass spectrometers are operated under vacuum to prevent the collision of ions with residual gas molecules during their flight from the ion source to the detector. This is because the ions are formed with excess energy and this, together with their charged character, can result in their reaction with other gaseous material present. To avoid this, the levels of contaminants and atmospheric gases such as oxygen within the ion source should be minimised. The ideal operating pressure is that in which the average distance an ion travels before colliding with a gas molecule (its *mean free path*) is longer than the distance from the source to the detector.

After ions are formed in the source, they are accelerated into the *mass analyser* where they are separated *in vacuo* according to their mass and charge through the use of electric and/or magnetic fields. Finally, the ions are passed onto an *ion detector* producing an electrical current that is amplified and detected.

In most mass spectrometers, these three basic components are physically discrete entities. Thus each of them will be considered separately in order to understand how ions are formed, separated and detected in mass spectrometry experiments. It is not possible to review all the ionisation techniques that have been used in mass spectrometry experiments, some of which have been replaced by other more efficient methods.

Mass Spectrometry: A Foundation Course, K. Downard
© Royal Society of Chemistry 2004

Downloaded from http://books.rsc.org/books/monograph/chapter-pdf/1169463/bk9780854046096-00022.pdf by Ecole Polytechnique Federale de Lausanne user on 16 May 2025

Instead, the following section discusses those most widely used approaches either alone, or in conjunction with, chromatographic and electrophoretic separations.

3.2 IONISATION TECHNIQUES AND INTERFACES

3.2.1 Electron Ionisation

As described in Section 2.1, the traditional method of ion production in mass spectrometry is *electron impact* or *electron ionisation* (EI) in which gaseous sample molecules are bombarded with a stream of electrons (equation 2.1). Other processes by which ions can be formed during electron ionisation include *dissociative ionisation* (equation 3.1), *ion pair formation* (equation 3.2) and *electron capture* in which negatively charged ions are produced (equation 2.2). The electron ionisation technique is widely used for the study of relatively volatile organic molecules by mass spectrometry.

$$AB + e^- \rightarrow A^{+\bullet} + B + 2e^- \quad (3.1)$$

$$AB + e^- \rightarrow A^+ + B^- + e^- \quad (3.2)$$

The electrons are produced by heating and passing a current through a thin ribbon of metal (such as ruthenium) known as the filament or cathode. The electrons are projected across the ion source by their attraction to an anode on the opposite side of the chamber (Figure 3.1). The energy of the electrons depends on the difference in the potentials applied to the cathode and anode. If a voltage difference of 70 V is maintained, the electrons have energies of 70 electron volts (eV), or the equivalent of 6.8×10^3 kJ mol^{-1}. A small magnetic field is usually applied across the ion source to cause the electrons to follow a helical path in order to increase the probability that they interact with a gaseous sample molecule. A pressure of typically 10^{-6} Torr or 1.3×10^{-4} Pa is maintained in the ion source.

The collision of electrons with the sample molecules, M, often leads to their ionisation by electron loss with the formation of the molecular ion, $M^{+\bullet}$ (equation 2.1). The energy required to ionise a molecule depends on the molecule itself but, as an example, an energy of 10.5 eV is needed to ionise a molecule of ethanol. Most organic molecules ionise in the range of 8–15 eV. Yet since not all the electron energy is necessarily transferred to the sample molecules during collision, higher electron energies of ~70 eV are commonly used in EI mass spectrometry.

Mass Spectrometry: A Foundation Course, K. Downard
© Royal Society of Chemistry 2004

Downloaded from http://books.rsc.org/books/monograph/chapter-pdf/1169463/bk9780854046096-00022.pdf by Ecole Polytechnique Federale de Lausanne user on 16 May 2025

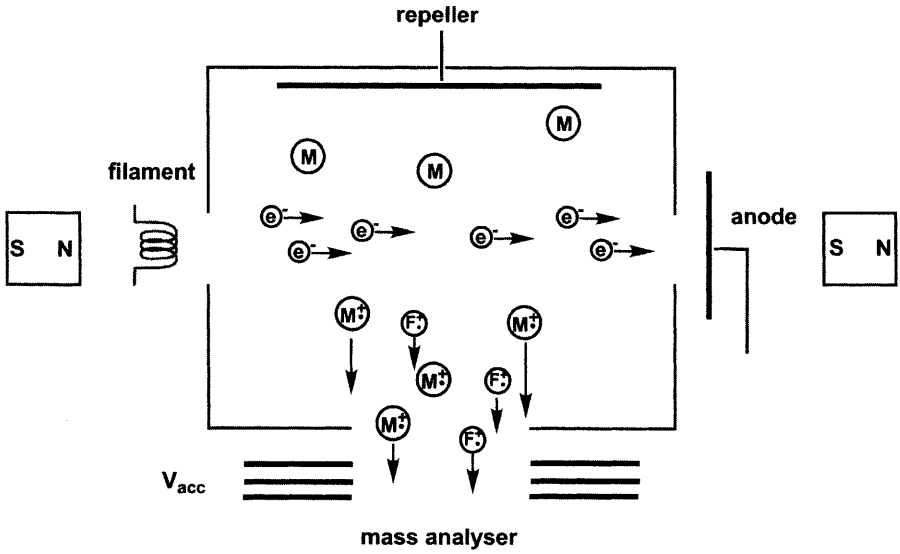

Figure 3.1 *Schematic representation of an electron ionisation source*

Regardless of the energy of the electrons, the sample molecules will possess a range of energies up to and including the energy of the electrons. Therefore some molecules will have sufficient energy to ionise (equations 2.1 and 2.2), others will not, and still others will contain enough energy to dissociate (equations 2.3, 2.4, 3.1, 3.2). Several factors contribute to the formation of fragment ions including the strength of the bonds to be broken, the stability of the products of fragmentation (both the ions and neutrals or radicals), and the internal energy of the fragment ions themselves. Where the dissociation of the molecular ions is problematic (and as shown in Chapter 5 it is often useful to determine the structure of a molecule), a lower potential difference (10–15 V) can be applied between the filament and the anode.

The voltage difference at which molecular ions are first observed in a mass spectrometer is known as the *ionisation potential*. A few volts above this, molecular ions are mostly formed. As the potential increases further, more fragment ions are produced (Figure 3.2).

In principle, mass spectrometers can be used to measure ionisation potentials. Because these measurements can be unreliable, a better measure is the efficiency of producing fragments. The *appearance potential* is related to the overall energy for the processes shown in equations 2.1, 2.3 and 2.4. This information is useful for measuring the thermodynamic parameters of a molecule such as its heat of formation and bond dissociation energies (see Chapter 6).

Mass Spectrometry: A Foundation Course, K. Downard
© Royal Society of Chemistry 2004

Downloaded from http://books.rsc.org/books/monograph/chapter-pdf/1169463/bk9780854046096-00022.pdf by Ecole Polytechnique Federale de Lausanne user on 16 May 2025

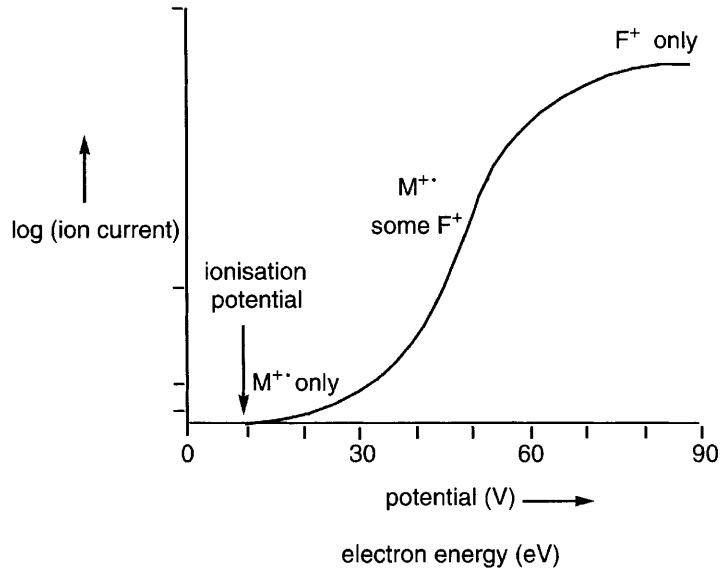

Figure 3.2 *Plot of ion current versus the potential difference between the filament (cathode) and anode. The ionisation potential represents the voltage at which molecular ions are first detected*

Since electron ionisation can lead to the production of fragments (F^+) (equation 2.3) as well as intact molecular ions, it is referred to as a *hard ionisation* method. The remaining ionisation methods discussed in this chapter result predominantly in the formation of ions without fragmentation, and hence are known as *soft ionisation* techniques.

3.2.2 Chemical Ionisation

Chemical ionisation (CI) is related to the electron impact method except that ionisation of a reagent gas, rather than the sample molecule itself, occurs first. This is followed by the transfer of charge to the sample molecule by a chemical process. One of the most common reagent gases is methane. When subjected to electron impact, a molecule of methane can ionise to form $CH_4^{+\bullet}$ by electron loss. This ion can react with a second molecule of methane, to produce CH_5^+ (equation 3.3).

$$CH_4^{+\bullet} + CH_4 \rightarrow CH_5^+ + CH_3^{\bullet} \qquad (3.3)$$

The ion CH_5^+ is an efficient proton donor, so that a sample molecule M also present in the ionisation chamber can be ionised according to equation 3.4.

$$M + CH_5^+ \rightarrow [M + H]^+ + CH_4 \qquad (3.4)$$

Mass Spectrometry: A Foundation Course, K. Downard
© Royal Society of Chemistry 2004

Downloaded from http://books.rsc.org/books/monograph/chapter-pdf/1169463/bk9780854046096-00022.pdf by Ecole Polytechnique Federale de Lausanne user on 16 May 2025

To prevent the direct ionisation of molecules M, methane is present in the ion source at a much higher concentration than the sample. Because of this, a chemical ionisation source operates at a much higher pressure (10^{-3} to 10^{-4} Torr, or 0.1 to 1 Pa) than an EI source.

There are many chemical processes other than proton transfer that can be achieved inside an ion source to effect chemical ionisation. These include *charge transfer* (or *charge exchange*) (equation 3.5), ion-molecule addition (equation 3.6), and even nucleophilic displacement reactions in the case of negatively-charged ions (equation 3.7).

$$M^{+\bullet} + N \rightarrow M + N^{+\bullet} \quad (3.5)$$

$$M^{+} + N \rightarrow [M + N]^{+} \quad (3.6)$$

$$Nu^{-} + AB \rightarrow Nu\text{-}A + B^{-} \quad (3.7)$$

3.2.3 Coupling Gas Chromatography to Mass Spectrometry (GC-MS)

The ability to ionise volatile molecules within a mass spectrometer led to mass spectrometers being coupled to gas chromatographs (GC). In these experiments, the volatile components of relatively complex sample mixtures can be ionised (by either electron or chemical ionisation) and detected in a stepwise manner as they are released from the GC-column. Modern GC-MS mass spectrometers use capillary columns (with 100–500 μm internal diameters) that provide for the separation of low levels of analytes and also minimise the amounts of GC carrier gas that enter the ion source. This helps to maintain a low operating pressure in the source. Modern GC-MS interfaces are designed to further minimise the levels of carrier gas that enter the ion source by using high capacity pumping systems. However, it is a requirement that some compromise be made between optimal GC conditions and those required for MS operation. A so-called *open-split interface* in which a proportion of the eluent from the column is pumped away before it enters the ion source is one way to achieve the coupling of a GC and mass spectrometer. This interface also enables the GC column to be interchanged without breaking vacuum ("venting") to the mass spectrometer.

3.2.4 Field and Plasma Desorption Ionisation

These two ionisation techniques have largely been superseded by other methods, but they are mentioned briefly here since they represent the first

Downloaded from http://books.rsc.org/books/monograph/chapter-pdf/1169463/bk9780854046096-00022.pdf by Ecole Polytechnique Federale de Lausanne user on 16 May 2025

Mass Spectrometry: A Foundation Course, K. Downard
© Royal Society of Chemistry 2004

methods available for ionising non-volatile molecules. Field desorption (FD) ionisation is achieved by depositing the sample onto a metal filament coated with carbon. A potential difference is applied between the filament and a nearby electrode such that ions are desorbed from the surface. The method is particularly useful for large non-polar compounds such as hydrocarbons but requires some skill to correctly prepare the coated filament.

In plasma desorption (PD) the sample is deposited onto a foil constructed of nickel or aluminium-coated nylon. The fission fragments from the radioactive decay of californium-252 then pass through the foil. Californium-252, an isotope with a half-life of about 2.6 years, is a very efficient neutron source with one microgram producing about 170 million neutrons every minute. The fission particles deposit considerable energy into the sample and lead to the direct release of ionised forms of the sample molecules, usually $[M + H]^+$ and $[M + Na]^+$ ions.

Plasma desorption (PD) ionisation was the first ionisation technique capable of ionising non-volatile sample molecules with molecular weights of the order of 10,000 Da including polar molecules such as proteins. However, the use of a radioactive source and difficulties with preparing the sample for ionisation led to it being largely replaced by fast atom bombardment (FAB) ionisation shortly after its discovery.

3.2.5 Fast Atom or Ion Bombardment

Fast atom bombardment (FAB) is related to an approach widely used to study the chemical nature of materials and surfaces called *secondary ion mass spectrometry* (SIMS) (see Chapter 9). In SIMS, a primary beam of high-energy (typically 10–30 keV) ions such as Xe^+ or Cs^+ bombard a solid surface, releasing secondary sample ions for analysis. One problem with the approach is that once the sample is ablated from a position on the surface, the yield of secondary ions decreases unless the primary ions are made to strike a different position.

To overcome this, Michael Barber and colleagues invented the FAB approach in which the sample compound was suspended or dissolved in a non-volatile viscous liquid. Once a portion of the sample is ablated or sputtered from the surface by a primary beam of atoms or ions, the liquid "matrix" flows back across this region restoring a proportion of sample (Figure 3.3). The matrix also serves an additional role by dissipating the energy from the primary beam to minimise molecular damage to the sample.

Mass Spectrometry: A Foundation Course, K. Downard
© Royal Society of Chemistry 2004

Downloaded from http://books.rsc.org/books/monograph/chapter-pdf/1169463/bk9780854046096-00022.pdf by Ecole Polytechnique Federale de Lausanne user on 16 May 2025

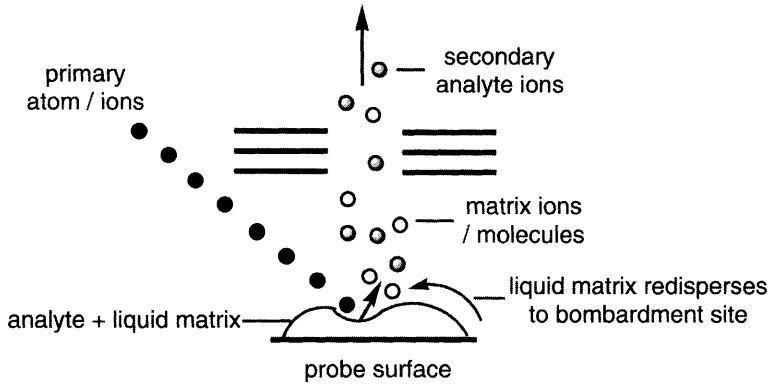

Figure 3.3 *Schematic representation of a fast atom/ion bombardment (FAB) source*

The original application of the FAB technique made use of high-energy atoms (rather than ions) of argon to bombard the sample dissolved in glycerol. These atoms are formed from a gas held at a relatively high pressure (10^{-3} to 10^{-4} Torr, or 0.1 to 1 Pa) in an *atom gun* by a charge exchange process with ionised gas (equations 3.8 and 3.9).

$$Ar + e^- \rightarrow Ar^{+\bullet} + 2e^- \quad (3.8)$$

$$Ar^{+\bullet}\ (fast) + Ar\ (slow) \rightarrow Ar\ (fast) + Ar^{+\bullet}\ (slow) \quad (3.9)$$

When the primary atoms or ions collide with the liquid surface, a charge is transferred to the involatile liquid matrix (M) molecules and subsequently to the analyte (A) sample molecules (equations 3.10–3.12).

$$Ar^{+\bullet} + M \rightarrow M^{+\bullet} + Ar \quad (3.10)$$

$$M^{+\bullet} + MH \rightarrow MH^+ + M^\bullet \quad (3.11)$$

$$MH^+ + A \rightarrow AH^+ + M \quad (3.12)$$

Thus secondary ions are produced from the liquid matrix ($M^{+\bullet}$ and MH^+) as well as the sample analyte (usually AH^+). Because the concentration of the former is much higher on the probe surface, FAB spectra have a characteristic high "background" at low to modest *m/z* ratios (up to 500 u) associated with ions of individual matrix molecules and their molecular clusters. This matrix background can obscure the detection of analyte ions below *m/z* 500 particularly where the levels of sample present are low. A representative early FAB mass spectrum for the peptide met-lys-bradykinin (MW 1318 Da) is shown in Figure 3.4.

Mass Spectrometry: A Foundation Course, K. Downard
© Royal Society of Chemistry 2004

Downloaded from http://books.rsc.org/books/monograph/chapter-pdf/1169463/bk9780854046096-00022.pdf by Ecole Polytechnique Federale de Lausanne user on 16 May 2025

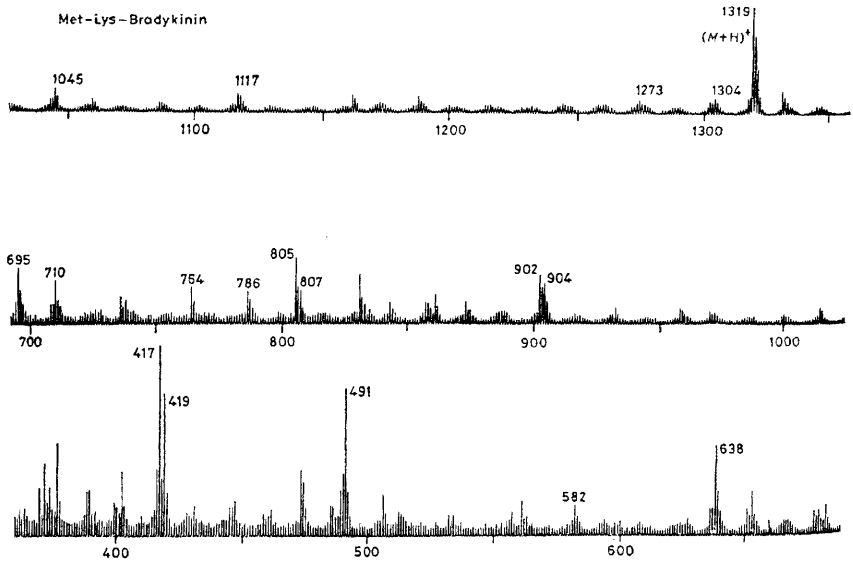

Figure 3.4 *FAB mass spectrum of the peptide met-lys-bradykinin*
(Source: M. Barber, R.S. Bordoli, G.J. Elliott, A.N. Tyler, J.C. Bill and B.N. Green. Fast atom bombardment (FAB) mass spectrometry: A new ion source for mass spectrometry, *J. Chem. Soc. Chem. Commun.*, 1981, 325–327, Figure 1)

Several properties of the liquid matrix are important to the success of the FAB method. The matrix should be an involatile liquid in vacuum, it should be chemically inert, and preferably dissolve most analytes. FAB matrices often possess a low *pKa* to assist in the generation of positively-charged AH^+ ions, or a high *pKa* where negatively-charged ions are to be produced. Glycerol, nitrobenzyl alcohol, thioglycerol and dimethylsulphoxide are common FAB matrices. Dithiothreitol and dithioerythritol can be added to the matrix thioglycerol (*magic bullet*) to reduce disulphide bonds in proteins suspended or dissolved in the matrix. Ions are typically produced by FAB ionisation over a period of several minutes before it is necessary to replenish the liquid matrix and/or the sample. Primary ions such as Cs^+ have largely replaced high-energy argon atoms in most FAB experiments, and thus the experiments have been referred to as liquid SIMS (LSIMS) in some accounts.

Samples can also be introduced directly from a high-pressure liquid chromatograph (HPLC) by means of continuous flow FAB. This technique involves adding the FAB matrix to the mobile liquid phase used in the HPLC experiment at approximately 5% by volume. The solution is pumped down the length of the FAB inlet probe onto a target (Figure

Mass Spectrometry: A Foundation Course, K. Downard
© Royal Society of Chemistry 2004

Downloaded from http://books.rsc.org/books/monograph/chapter-pdf/1169463/bk9780854046096-00022.pdf by Ecole Polytechnique Federale de Lausanne user on 16 May 2025

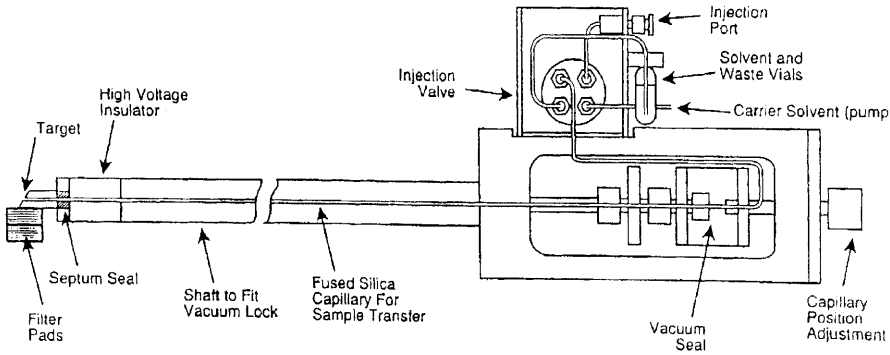

Figure 3.5 *Design of a continuous flow CF-FAB sample probe*
(Source: R.M. Caprioli and W.T. Moore, in *Methods in Enzymology*, McCloskey (ed), Academic Press, New York, 1990, Vol. 193, Ch. 9, p. 216, Figure 1)

3.5) or mesh frit. A filter below the probe tip is used to absorb excess matrix. Stable ion signals are produced when the amount of liquid delivered to the tip or frit is equal to the rate of evaporation. The reduced concentration of matrix on the tip over standard or static FAB leads to a lower background of matrix ions that can aid the detection of analyte ions.

Although FAB ionisation is less practiced today due to the development of laser based and spray ionisation methods, it remains an important technique for many applications. It is still in use for the study of organic and smaller biological compounds in many laboratories.

3.2.6 Laser Desorption and MALDI

Lasers operating in both the ultraviolet (UV) and infrared (IR) have been used to desorb and ionise samples from solid surfaces for some time. The transfer of energy from the laser pulse leads to electronic excitation of the sample. Laser powers vary from approximately 10^6 to 10^{10} J sec^{-1} cm^{-2} where the total energy per pulse is of the order of a few millijoules to a joule. Apart from the region of the sample upon which the laser is focused, there is usually little excess energy dissipated through the analyte. That said, considerable decomposition of some analytes can occur following the laser pulse. Despite this, a number of moderately-sized (~1000 Da) sample molecules such as oligosaccharides, peptides and polymers have been successfully ionised by laser desorption ionisation (LDI, or just LD). This "neat" desorption strategy was effectively replaced with the development of *matrix-assisted laser desorption ionisation* (MALDI).

Mass Spectrometry: A Foundation Course, K. Downard
© Royal Society of Chemistry 2004

Downloaded from http://books.rsc.org/books/monograph/chapter-pdf/1169463/bk9780854046096-00022.pdf by Ecole Polytechnique Federale de Lausanne user on 16 May 2025

In MALDI, the analyte of interest is mixed with a large mole excess of (*ca.* 1,000-fold) a matrix compound that absorbs efficiently at the laser wavelength. The matrix allows the energy from the laser to be dissipated and also assists with the ionisation sample molecules through electron transfer and chemical processes. Both solid and liquid matrices have been used, though the former is by far the more successful in most applications.

Where a UV laser is used, common MALDI matrices include nicotinic acid, 2,5-dihydroxybenzoic acid, sinapinic acid and α-cyano-4-hydroxycinnamic acid (Figure 3.6). It is important to note that all of the MALDI matrices in Figure 3.6 contain phenolic and/or carboxylic acid groups. It has been found that proton transfer from matrix to sample molecules is important in order to achieve efficient ionisation of many compounds and that this transfer occurs, at least in part, in the vapour phase above the sample plate.

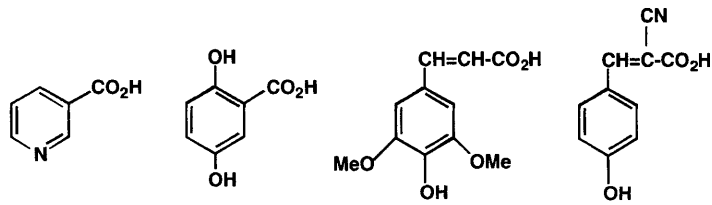

Figure 3.6 *MALDI matrices nicotinic acid, 2,5-dihydroxybenzoic acid, sinapinic acid and α-cyano-4-hydroxycinnamic acid (in order from left to right)*

The correct preparation and deposition of MALDI samples onto the sample plate is critical to the success of the method. Most sample solutions are diluted in a solution of matrix, and a small volume of the combined solution (1 μl or less) is deposited onto the sample plate. The sample droplet is usually allowed to dry in ambient air (by so-called *dried droplet* evaporation). However, since the morphology of the crystallised sample surface affects the success of MALDI mass spectrometry, other methods to deposit samples have been developed. These include the addition of organic solvents to the solution, the use of heat to assist the drying process, and the electrostatic-spraying of solutions of analyte and matrix (either separately or in a combined form). The latter technique gives rise to extremely thin and uniform surface of both the analyte and matrix resulting in more reproducible mass spectra being obtained regardless of the position from which the sample is ablated by the laser.

A further useful strategy that has been adopted where the concentrations of analyte are low is the deposition of droplets onto pre-coated sample surfaces to either localise (for example, through the use of

Mass Spectrometry: A Foundation Course, K. Downard
© Royal Society of Chemistry 2004

Downloaded from http://books.rsc.org/books/monograph/chapter-pdf/1169463/bk9780854046096-00022.pdf by Ecole Polytechnique Federale de Lausanne user on 16 May 2025

Teflon-based surfaces) or immobilise the analyte molecules onto a very small sample area (<1 mm^2). Subsequent chemistries can also be performed on the molecules on these surfaces prior to their analysis by mass spectrometry. One such MALDI-based approach has been dubbed SELDI for *surface-enhanced laser desorption ionisation.*

Since the laser or sample surface can be easily repositioned during analysis, most MALDI-based mass spectrometers today make use of a sample stage onto which 100 to several hundred samples are loaded. The plates also resemble the size of a microgel or blot (some 10 cm^2) to facilitate the direct transfer of samples (particularly proteins) after their separation by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). Greater success has been achieved in experiments where the proteins are blotted onto a membrane to which a solution of matrix is applied. Unfortunately, the direct ionisation of proteins from gels has proved more difficult due to the presence of detergents and other contaminants that impede the ionisation process.

A representative MALDI mass spectrum for a protein mixture is shown in Figure 3.7. Consistent with most protein analyses, the dominate ions in the spectrum have the form $[M + H]^+$ from which a molecular weight can easily be derived. Note in this case that insufficient mass resolution has been obtained in this case to resolve the isotopes for the $[M + H]^+$ ions.

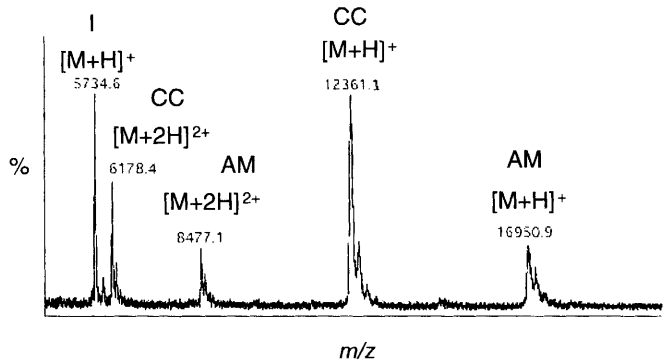

Figure 3.7 *Linear MALDI-TOF mass spectrum of the protein mixture, insulin (I), cytochrome c (CC) and apomyoglobin (AM)*

MALDI is now a firmly established technique, particularly for the study of polar, high molecular weight compounds such as proteins, glycoconjugates and nucleic acids. In most mass spectrometry laboratories today, its use is complemented by the application of *electrospray ionisation* (ESI), a completely unrelated ionisation method described in the next section.

Mass Spectrometry: A Foundation Course, K. Downard
© Royal Society of Chemistry 2004

Downloaded from http://books.rsc.org/books/monograph/chapter-pdf/1169463/bk9780854046096-00022.pdf by Ecole Polytechnique Federale de Lausanne user on 16 May 2025

3.2.7 Spray Ionisation Methods; Thermospray

One disadvantage of MALDI, and other ionisation methods, in which the sample is deposited in its solid state is the difficulty in performing high throughput separations in conjunction with mass spectrometric analysis. Although mass spectrometry can be used to analyse sample mixtures directly, some components may not be mass resolved and thus not be detected as the complexity of these mixtures increases.

Various chromatographic and electrophoretic approaches are used widely for the separation of components within complex chemical and biological extracts. The development of solution-based spray ionisation approaches have enabled these technologies to be coupled directly to a mass spectrometer providing a further separation dimension prior to MS analysis.

In 1983, Blakley and Vestal reported the development of the *thermospray ionisation* method for this purpose. Briefly, a dilute solution of an analyte is pumped through a stainless steel tube and heated to approximately 100 °C subject to the flow rate and nature of the solution. A jet of vapour containing a mist of solution droplets is projected into the ion source by a free jet expansion and preformed ions in solution are evaporated and detected. Thermospray ionisation has been applied to the study of small organic molecules and moderately-sized biological molecules such as peptides but has been largely superseded by electrospray ionisation.

3.2.8 Electrospray Ionisation

Electrospray ionisation (ESI) was first conceived in the late 1960s by Malcolm Dole and has developed from experiments performed in the late 1980s by John Fenn and colleagues. The electrostatic spraying of liquids is used in many industrial applications and involves passing a solution through a needle held at high voltage (typically 4–5 kV) relative to some counter electrode. When the solution is an electrolyte and the needle forms part of an ion source in a mass spectrometer, the fine mist of droplets that emerge from the needle tip possess a net positive or negative charge determined by the polarity of the needle and are attracted to the entrance of a mass analyser (Figure 3.8). The droplets emerge from what is known as a *Taylor cone* formed by the elongation of the electrolyte solution at the needle tip as like-charged ions are repelled from the needle. The application of a "counter-current" dry gas (that passes in the opposite direction to the passage of droplets) considerably aids droplet evaporation and it was this feature that led to the successful ionisation of large molecules.

Mass Spectrometry: A Foundation Course, K. Downard
© Royal Society of Chemistry 2004

Downloaded from http://books.rsc.org/books/monograph/chapter-pdf/1169463/bk9780854046096-00022.pdf by Ecole Polytechnique Federale de Lausanne user on 16 May 2025

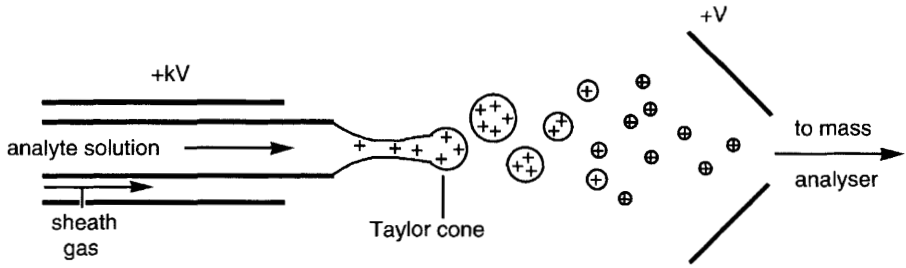

Figure 3.8 *Magnified representation of the cross-section of an electrospray ion source.*

As the droplets evaporate, the ions within them move closer together. At some point there are sufficient Coulombic repulsive forces between the ions to overcome the liquid surface tensions, resulting in the production of smaller droplets that continue to undergo the process. Eventually solvent-free ions are produced that are passed through the mass analyser and detected.

A curious feature of the electrospray ionisation mass spectra of large biopolymers is that the ions produced are usually multiply-charged, with a continuous series of such ions being detected (Figure 3.9). The reasons for this are not entirely clear though the phenomenon has been linked to the time it takes for ions to emerge from the solution droplets during the evaporation process.

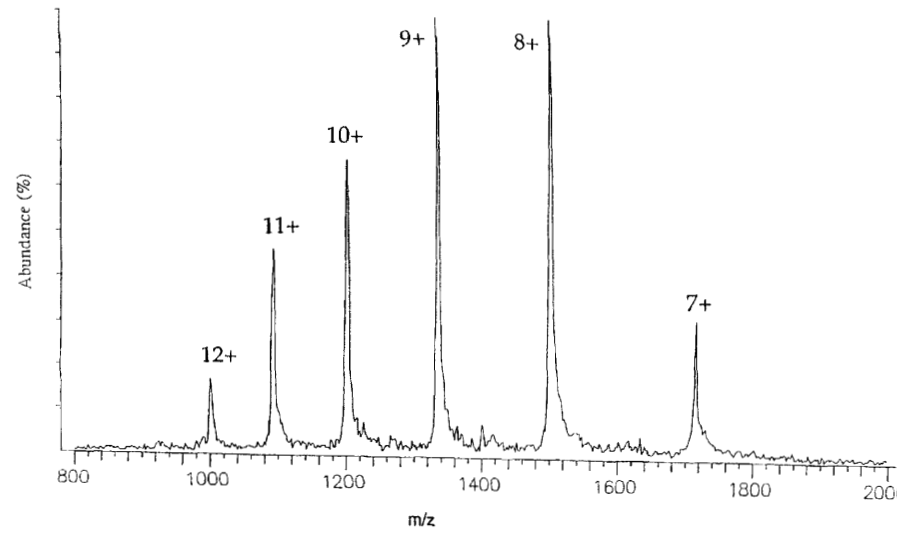

Figure 3.9 *Electrospray ionization (ESI) mass spectrum of protein chloroflexus thioredoxin exhibiting ions of the form* $[M+nH]^{n+}$
(Source: K.M. Downard, Advances in protein analysis and sequencing by mass spectrometry, *New Adv. Anal. Chem.*, 2002, **P2**,1–30, Figure 1 (adapted))

Mass Spectrometry: A Foundation Course, K. Downard
© Royal Society of Chemistry 2004

Downloaded from http://books.rsc.org/books/monograph/chapter-pdf/1169463/bk9780854046096-00022.pdf by Ecole Polytechnique Federale de Lausanne user on 16 May 2025

An advantage of this feature is that a molecular weight measurement can be made based on each multiply-charged ion and the values averaged across all charge states. This leads to routine mass accuracies of ± 0.01%, or a 1 Da error at a molecular weight of 10,000 Da even on mass spectrometers with modest mass resolving capabilities. To achieve this, the charge state for a particular ion must be determined. If it is assumed that two neighbouring ions (with mass-to-charge ratios of m_i/z_i and m_j/z_j where $m_i/z_i < m_j/z_j$) differ by one charge unit and both support the same charge-bearing species, then the charge on ion i (z_i) is given by equation 3.13 where m_p is the mass of the charge-bearing ion, typically a proton.

$$z_i = (m_j/z_j - m_p)/(m_j/z_j - m_i/z_i) \tag{3.13}$$

Once the charge of any ion is derived, a molecular weight measurement based on any ion signal can be determined from equation 3.14.

$$\text{MW} = z_i\,(m_i/z_i) - z_i\,m_p \tag{3.14}$$

Applying this equation in the case of the data presented in Figure 3.9, the charge of the protonated ions at *m/z* 1,335.4 is $z = (1{,}502.2-1)/(1{,}502.2-1{,}335.4) = 9.0$. The molecular weight of the protein based on this protonated ion signal is thus $9(1{,}335.4) - 9(1.0) = 12{,}009.6$. The same molecular weight value is obtained based on the *m/z* for the $[M + 8H]^{8+}$ ion; that is $8(1{,}502.2) - 8(1.0) = 12{,}009.6$.

Several computer algorithms have been developed to perform these calculations automatically and to display the output on a molecular weight scale in what has become known as a *deconvoluted mass spectrum*. One such algorithm considers the mass of the charge-bearing ions as a variable and constructs a three-dimensional deconvoluted mass spectrum from which the identity of the charge-bearing species can be determined and not assumed.

3.2.9 Atmospheric Pressure Chemical Ionisation

A related approach that is also capable of ionising polar molecules directly from solution is known as *atmospheric pressure chemical ionisation* (APCI). In this method, ionisation is achieved by an electrical discharge in the vicinity of gaseous sample molecules produced by vapourising the solution stream. Ionisation takes place through chemical processes such as those described in Section 3.2.2. The process is less

Mass Spectrometry: A Foundation Course, K. Downard
© Royal Society of Chemistry 2004

Downloaded from http://books.rsc.org/books/monograph/chapter-pdf/1169463/bk9780854046096-00022.pdf by Ecole Polytechnique Federale de Lausanne user on 16 May 2025

efficient at ionising large molecular weight compounds, but does have utility for many modestly-sized polar biomolecules (to ~ 1000 Da). For this reason, the approach is employed widely in drug discovery investigations including the study of metabolites (see Chapter 8). It works well even for relatively high flow rates (several ml min^{-1}) that are common in analytical HPLC applications. Since the ESI and APCI ionisation approaches are related and have complementary applications, many instruments feature interchangeable ESI and APCI ion sources.

A disadvantage of ESI mass spectra of large compounds is that many ions are associated with each component present in the sample solution. In the case of a complex sample mixture, these ion distributions could be incorrectly associated with one another so that molecular weight errors arise. However, to overcome this the ionisation method can be coupled to a liquid chromatograph or capillary electrophoresis system where some initial separation of components is effected prior to MS analysis.

3.2.10 Coupling Liquid Chromatography and Capillary Electrophoresis with Mass Spectrometry

There are two major considerations in coupling liquid chromatography and capillary electrophoresis separation systems to a mass spectrometer. First, the solvent must be efficiently evaporated before ions leave the source to enable the pressure within the mass analyser to be maintained. This also aids the efficient sampling of ions. Second, the nature of the solvent and other dissolved components (buffers, salts, denaturants *etc.*) should not impede the ionisation process. There is clearly a compromise reached in order to optimise the separation of chemical and biological mixtures with their direct on-line detection by mass spectrometry. Nonetheless, reproducible data can be acquired when appropriate operating conditions are maintained.

A typical liquid chromatographic ESI mass spectrometry (LC-ESI-MS) apparatus is illustrated in Figure 3.10. In brief, solvent pumped from the reservoirs enters the injector and passes through the chromatography column to the mass spectrometer. A UV detector can also be incorporated such that components are detected by absorption spectroscopy and mass spectrometry either simultaneously or in tandem. Once the sample is injected into the loop, the solvent delivers it to the column where chromatographic separation of the components occurs. The components then pass one by one, or as simpler mixtures, into the ion source where they are ionised and ultimately detected. Note that since the mass spectrometer separates the ionic forms by mass analysis, it is not

Mass Spectrometry: A Foundation Course, K. Downard
© Royal Society of Chemistry 2004

Downloaded from http://books.rsc.org/books/monograph/chapter-pdf/1169463/bk9780854046096-00022.pdf by Ecole Polytechnique Federale de Lausanne user on 16 May 2025

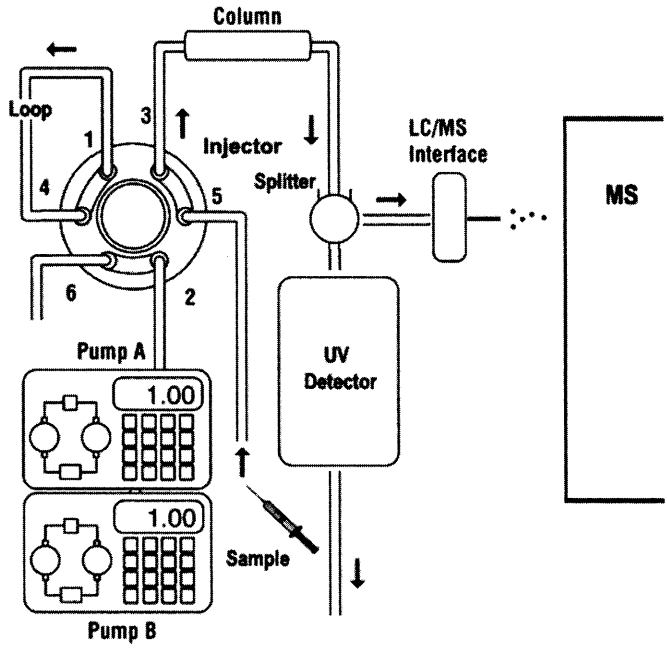

Figure 3.10 *Representation of a liquid chromatographic ESI mass spectrometry (LC-ESI-MS) experiment*

necessary that complete chromatographic separation of each component of a mixture be achieved. Thus the chromatographic separation step is less rigorous than that which would be required where no mass spectrometer was employed.

In the case of liquid chromatography, solvent flow rates of the order of several nanolitres to millilitres per minute have been coupled to ESI and APCI sources. An approximately linear correlation between the yield of ions detected and the concentration of the analyte in solution is observed over several orders of magnitude. Thus a quantitative measure of the ion current for each component enables the relative concentration of the analytes in solution to be determined. The addition of standards of known concentration into the sample, or their analysis in a separate run, allows such LC–MS approaches to be used for quantitative applications.

The preferred mobile phases are those that contain significant levels of a volatile organic solvent such as acetonitrile or methanol, both of which are widely used for chromatographic separations. Pure aqueous solvent systems, however, can also be managed albeit with slightly reduced performance. The presence of low levels of ion pairing agents such as trifluoroacetic acid can assist with generating preformed ions of the

Mass Spectrometry: A Foundation Course, K. Downard
© Royal Society of Chemistry 2004

Downloaded from http://books.rsc.org/books/monograph/chapter-pdf/1169463/bk9780854046096-00022.pdf by Ecole Polytechnique Federale de Lausanne user on 16 May 2025

sample components in solution. High levels of salt and other buffers and denaturants, however, should be diverted away from the ion source, usually during the early stages of the run, in order to prevent their build up in the transfer lines and on the spray needle.

One further issue in performing such experiments on most mass spectrometers is whether or not all ions across the *m/z* range of the mass analyser are detected simultaneously. Where mass analysers are used in which the electric and/or magnetic fields are scanned, ions produced from an analyte eluting over a relatively small time period may pass into the mass analyser but not be transmitted to the detector. To compensate (if not correct) for this, either fast scanning of the mass analyser is employed or the flow rate of the mobile phase is reduced.

An alternate method to detect low levels of compounds in these experiments is to "park" the electric and/or magnetic fields of the mass analyser to transmit only ions of a particular *m/z* ratio onto the detector. This *selected ion monitoring* (SIM) mode is useful for quantitative analysis of particular components in chemical and biological samples.

Capillary electrophoresis mass spectrometry (CE-MS) is achieved in much the same way as that outlined in Figure 3.10. Capillary electrophoresis separates charged compounds with high resolution and is compatible with ESI-MS. Problems with coupling capillary electrophoresis with a mass spectrometer stem from the relatively high levels of buffers and salts used that can clog the transfer lines and disrupt the ionisation process. Gel-filled capillaries can be utilised to remove buffers from the ion source. Alternatively, narrow bore (5–10 μm) capillaries are used to reduce the electrolyte flow. The use of low flow rates (nl min^{-1}) in general offers improved sensitivities that are exploited in many applications of electrospray ionisation mass spectrometry.

3.2.11 Low Flow Rate Electrospray Ionisation – Nanospray

Electrospray ionisation mass spectrometry is most often performed using solution flow rates of several μl min^{-1}. There are several advantages, however, in using considerably lower flow rates (10–50 nl min^{-1}). These include compatibility with micro-flow LC and CE separation, improved spray stability, and lower sample consumption. Such experiments can also be performed without the use of a sheath gas passing around the spray needle to direct the electrosprayed droplets. Since there is also little solvent to evaporate during the ionisation process,

Downloaded from http://books.rsc.org/books/monograph/chapter-pdf/1169463/bk9780854046096-00022.pdf by Ecole Polytechnique Federale de Lausanne user on 16 May 2025

Mass Spectrometry: A Foundation Course, K. Downard
© Royal Society of Chemistry 2004

heating the source chamber and/or the use of counter-current dry gases can be avoided.

These so-called *micro-electrospray* or *nanospray* experiments can also be performed off-line by spraying liquids from microfine capillaries prepared by etching or drawing out glass capillaries (Figure 3.11). The capillary is loaded with approximately 1 μl of analyte solution and the flow rate is maintained by the electrospray process without the need for a delivery device such as a syringe pump. The capillary is either coated with a conducting material or a non-corrosive conductive wire is passed through the capillary to supply the high voltage to the tip. A camera or microscope can be used to position the capillary a few millimetres from the entrance lens to the mass analyser.

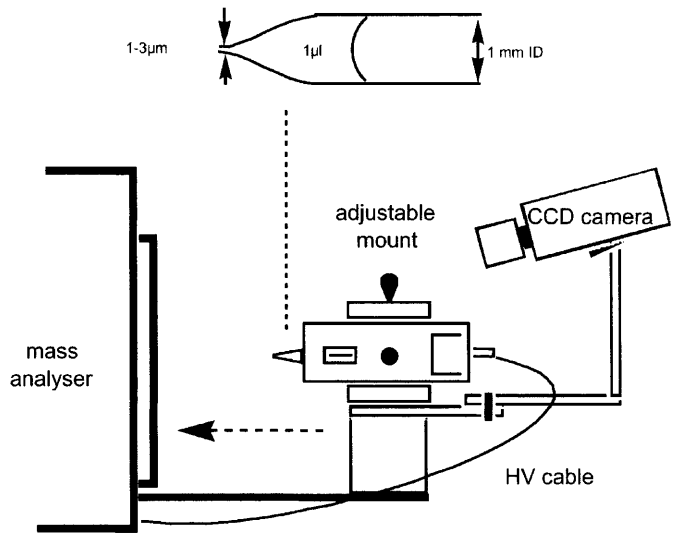

Figure 3.11 *Nanospray needle is mounted on an adjustable support for positioning a few millimetres from the entrance to the mass analyser*

A comparison of the common ionisation techniques is presented in Appendix 3. Once molecules have been introduced into a mass spectrometer as their ions, a mass analyser is used to guide them to the detector through the application of electric and magnetic fields.

3.3 MASS ANALYSERS

Once ions have been formed and introduced into a mass spectrometer, a mass analyser is used to separate them based upon their mass-to-charge ratio through the application of electric and magnetic fields. There are a

Mass Spectrometry: A Foundation Course, K. Downard
© Royal Society of Chemistry 2004

Downloaded from http://books.rsc.org/books/monograph/chapter-pdf/1169463/bk9780854046096-00022.pdf by Ecole Polytechnique Federale de Lausanne user on 16 May 2025

number of different mass analysers described in the following sub-sections. Many modern instruments feature several mass analysers coupled together for use in tandem mass spectrometry (see Chapter 4) and other applications. When a mass spectrometer is constructed of several mass analysers of a different type, it is referred to as a *hybrid* instrument. This section begins with the simplest mass analyser, the time-of-flight tube.

3.3.1 Time-of-Flight

As the name implies, time-of-flight (TOF) mass spectrometers separate ions and measure their *m/z* based on the time they take to pass ("fly") from the ion source to the detector. The flight tube is usually 1–2 m in length and the basis of the separation makes no use of either electric or magnetic fields. Ions are separated in the *field-free region* of the flight tube before reaching the detector. A simple representation of a TOF mass spectrometer is shown in Figure 3.12.

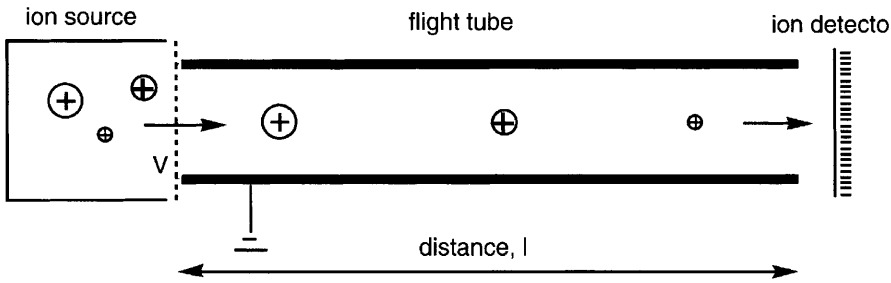

Figure 3.12 *Representation of a time-of-flight (TOF) mass spectrometer*

Ions are first formed in the source and then "pushed" down the flight tube through the application of a high accelerating potential (V) of the same polarity of the ions applied to a lens or grid. The kinetic energy of ions of mass, *m*, and charge, *z*, is given by equation 3.15 where *v* is their velocity. All like-charged ions (common *z*) share the same initial kinetic energy (KE) as they leave the ion source.

$$\mathrm{KE} = 1/2mv^2 = z\mathrm{eV} \tag{3.15}$$

The time, *t*, is takes for the ions to pass the length of the tube (*l*) is given by $t = l/v$. Substituting for *v* in equation 3.15 and rearranging, leads to equation 3.16.

$$t^2 = m/z\ (l^2/2\ eV) \tag{3.16}$$

Mass Spectrometry: A Foundation Course, K. Downard
© Royal Society of Chemistry 2004

Downloaded from http://books.rsc.org/books/monograph/chapter-pdf/1169463/bk9780854046096-00022.pdf by Ecole Polytechnique Federale de Lausanne user on 16 May 2025

Since the length of the flight tube (l) and accelerating voltage (V) are fixed, the time it takes for the ions to reach the detector depends only on their mass and charge. If the time it takes for at least two ions of known mass-to-charge ratio to reach the detector is measured, the time scale can be correlated with m/z values. As a general rule of thumb, singly-charged ions of molecules of 10,000 Da take about 100 μsec to reach the detector. Common accelerating voltages are of the order of 10–30 kV.

As one might expect, a flight tube of a common length (1–2 m) would not be able to separate ions with very similar mass-to-charge ratios. An additional complication arises since, due to the spatial distribution of ions in the ion source and their proximity to the applied electric field, not all the ions receive the same initial kinetic energy. These factors give rise to relatively poor mass resolutions of the order of 100–500 in linear TOF mass spectra. This leads to components in mixtures being unresolved from one another and large errors (~1%) in molecular weight measurements.

To overcome this, several features are now built into most TOF mass analysers that considerably improve mass resolution and thus mass accuracies. The first of these is an *ion mirror*, *ion reflector* or simply a *reflectron* (Figure 3.13). A reflectron is constructed of a stack of donut-shaped lens connected by a series of resistors across which a high voltage (V_R) is applied. In most instances the voltage difference between each lens of the stack is identical creating a linear or homogeneous field. So-called curved or inhomogenous field reflectrons have also been developed and have some advantages for transmitting ions across a wide m/z range. The potentials applied across the lenses of the reflectron causes the ions that enter it to be gradually repelled. These ions are reflected down the same or second flight tube to a second detector. Improvements in mass resolution are achieved because ions of different kinetic energies

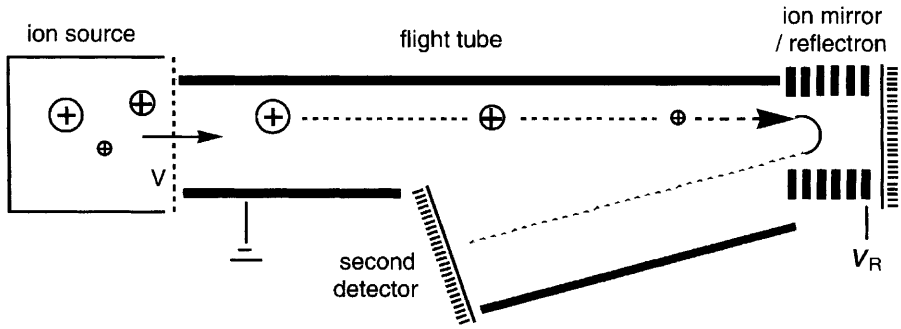

Figure 3.13 *Representation of a reflecting time-of-flight (rTOF) mass spectrometer*

Mass Spectrometry: A Foundation Course, K. Downard
© Royal Society of Chemistry 2004

Downloaded from http://books.rsc.org/books/monograph/chapter-pdf/1169463/bk9780854046096-00022.pdf by Ecole Polytechnique Federale de Lausanne user on 16 May 2025

penetrate the mirror to differing degrees. Furthermore, the reflectron effectively extends the flight tube to almost twice its length which (from equation 3.16) can be seen to have a dramatic effect on an ion's flight time. Note that the instrument can also be operated in a linear-mode since, where no voltage is applied to the reflectron, ions pass through it to the first detector.

Consider for the moment two ions of the same *m/z* that have slightly different initial kinetic energies, KE_1 and KE_2 (and velocities v_1 and v_2) when they leave the ion source, where $KE_1 > KE_2$. Ions with greater kinetic energy will pass further into the reflectron before being repelled, while ions with less kinetic energy will travel over a shorter distance. This difference in the flight path and time corrects for the differences in the kinetic energies of the ions so that they reach the detector at the same time.

A dramatic improvement in mass resolution is evident in TOF mass analysers operating in the reflectron over the linear mode as is illustrated for a segment (residues 18–39) of the peptide adrenocorticotropic hormone (ACTH) (Figure 3.14). The best mass resolution is achieved when ions spend equal times in the reflectron and the flight tube of the reflecting time-of-flight (RTOF) analyser.

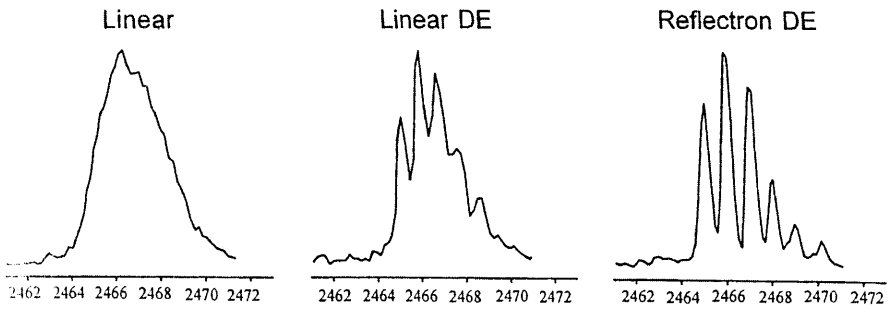

Figure 3.14 *TOF mass spectra of the $[M+H]^+$ ions of the peptide ACTH18–39 recorded in the linear (left), linear with delayed extraction (DE) (centre) and reflectron DE mode (right)*

A second feature to improve mass resolution that is employed today in most TOF instruments is *time-lag focusing* (TLF). Time-lag focusing has been revisited in recent years to improve the performance of MALDI-TOF experiments and has been described by different names including *delayed or pulsed-ion extraction* (DE or PIE). In conventional experiments, ions are extracted from the ion source through the application of an accelerating potential immediately after they are formed. If a time

Mass Spectrometry: A Foundation Course, K. Downard
© Royal Society of Chemistry 2004

Downloaded from http://books.rsc.org/books/monograph/chapter-pdf/1169463/bk9780854046096-00022.pdf by Ecole Polytechnique Federale de Lausanne user on 16 May 2025

delay is introduced before the application of this potential, ions formed with more initial kinetic energy and greater velocities will move further from the ion extraction lens or grid. Application of an accelerating potential pulse imparts more energy into the ions further from the lens than those closer to it. The amplitude is adjusted so that the initially less-energetic ions further from the lens will catch up to the initially more-energetic ions so that they all reach the detector at the same time (Figure 3.15). The improvement in mass resolution that can be attained is illustrated in Figure 3.14.

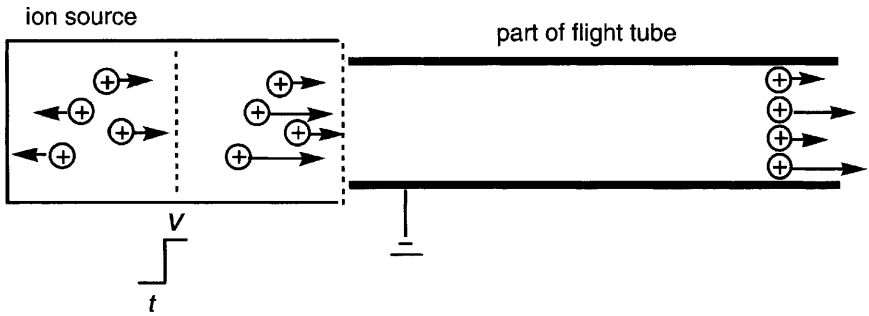

Figure 3.15 *Principle of time-lag focussing (TLF) on a time-of-flight mass spectrometer*

Mass resolutions of up to 30,000 (at FWHM) can now be achieved on TOF mass spectrometers by making use of both time-lag focusing and ion reflectrons. This is a dramatic improvement over their historical mass resolving capabilities. Consequently, TOF instruments can be described as analysers that achieve modest to high mass-resolutions that are second only to those of magnetic-based instruments.

3.3.2 Magnetic Sector

Instruments that contain a magnet positioned over one region of the ions' flight path are the oldest type of mass spectrometer. An ion of charge z moving with a velocity, v, that transverses a magnetic field B at right angles to the direction of the field will experience a centrifugal force given by $zevB$. When this force is equal to the centripetal force, ions adopt a circular path of radius r (equation 3.17).

$$zevB = (mv^2)/r \tag{3.17}$$

When equation 3.17 is rearranged, equation 3.18 is produced.

$$r = (mv)/zeB \tag{3.18}$$

Mass Spectrometry: A Foundation Course, K. Downard
© Royal Society of Chemistry 2004

Downloaded from http://books.rsc.org/books/monograph/chapter-pdf/1169463/bk9780854046096-00022.pdf by Ecole Polytechnique Federale de Lausanne user on 16 May 2025

This equation indicates that for ions of a particular charge z moving through a fixed magnetic field, B, the radius of their path is dependent only upon their momentum mv. In other words, ions of the same charge will follow a different path when they move pass the magnet influenced only by their mass and velocity (Figure 3.16).

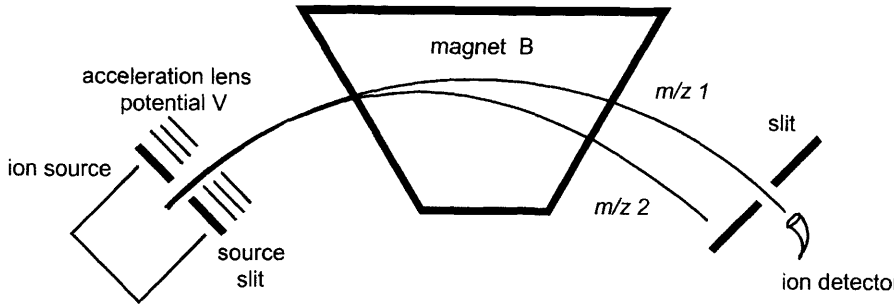

Figure 3.16 *Passage of ions of different m/z through a magnetic field of a single focusing mass spectrometer*

Since the initial kinetic energy of the ions $1/2mv^2$ equals zeV, the initial velocity of the ions is dependent on the potential, V, through which they are accelerated. Rearranging for v^2 we arrive at equation 3.19.

$$v^2 = (2zeV)/m \tag{3.19}$$

From equation 3.17 we can derive:

$$v = (zeBr)/m \tag{3.20}$$

If we square both sides of equation 3.20 we get:

$$v^2 = (zeBr)^2/m^2 \tag{3.21}$$

If equations 3.19 and 3.21 are combined and rearranged, we arrive at equation 3.22.

$$m/z = eB^2r^2/2V \tag{3.22}$$

Therefore specific values of V or B allow ions unique in mass-to-charge to pass through the magnetic field along a path to the detector. Variations in either V or B will cause these same ions to follow a different trajectory and collide with the walls of the flight tube. In Figure 3.16, only ions following the centre trajectory reach the detector at any set of

Mass Spectrometry: A Foundation Course, K. Downard
© Royal Society of Chemistry 2004

Downloaded from http://books.rsc.org/books/monograph/chapter-pdf/1169463/bk9780854046096-00022.pdf by Ecole Polytechnique Federale de Lausanne user on 16 May 2025

V and B values. In practice, a series of slits are used throughout the instrument to further improve the focusing and separation of ions.

It follows that a complete mass spectrum, in which all ions in turn are passed to the detector, can be recorded by changing (scanning) V or B over time. In practice, when the accelerating voltage is too low insufficient numbers of ions will leave the ion source and reach the detector. For this, and other reasons, scanning of the magnetic field B is preferred. However, the scan rate of this type of mass analyser is limited by *hysteresis* where the magnetic field can become perturbed. To minimise this, magnetics are scanned more slowly than other mass analysers with time allowed between scans to "settle" the field. Laminated magnetics, however, allow for more rapid scan rates, approaching 0.1 sec decade^{-1} (where a decade is equal to a range covering an order of magnitude difference in mass units, *e.g.* 100 to 1000 u), to be achieved.

As mentioned earlier, ions leave the source with a range of kinetic energies rather than a single value due to their spatial distribution. Since some ions of the same mass will have different velocities and will still reach the detector for a particular set of B and V values, the mass resolution achieved by a single magnet is compromised. To minimise this problem, most modern magnetic sector mass spectrometers also feature an electrostatic or electric sector.

If a radial electrostatic field E is created by two curved plates held at oppositely charged potentials ($+E$ and $-E$), an ion of charge z moving with a velocity v will transverse the field when its electrostatic force equals the centripetal force (equation 3.23).

$$zeE = (mv^2)/r \tag{3.23}$$

Since the kinetic energy of an ion $1/2mv^2$ equals zeV, equation 3.24 becomes:

$$r = 2V/E \tag{3.24}$$

Note that the trajectory of the ion defined by r is independent of its mass and charge. At a fixed accelerating voltage, V, the ion's trajectory is thus dependent only on the electric field strength.

Mass spectrometers that combine electric and magnetic sectors are known as *double-focusing* instruments. In most cases, the electric sector is positioned before the magnetic sector in terms of the direction that the ions travel (Figure 3.17). These instruments are referred to as *forward geometry* instruments. Mass spectrometers with the reverse order of sectors are termed *reverse geometry*. The order of mass analysers after

Downloaded from http://books.rsc.org/books/monograph/chapter-pdf/1169463/bk9780854046096-00022.pdf by Ecole Polytechnique Federale de Lausanne user on 16 May 2025

Mass Spectrometry: A Foundation Course, K. Downard
© Royal Society of Chemistry 2004

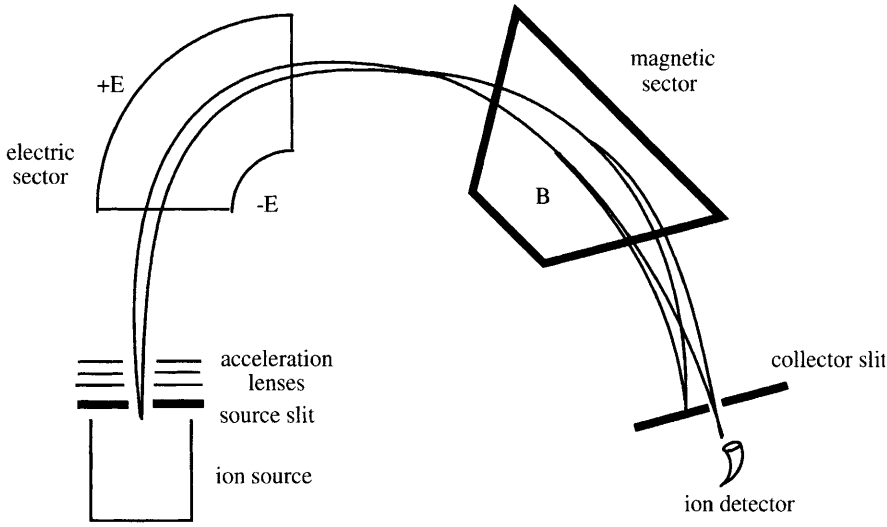

Figure 3.17 *Passage of ions through a double focusing (EB) magnetic sector mass spectrometer*

the ion source is often abbreviated simply as EB or BE. Mass spectrometers can be constructed of even more (three, four, five and even six!) sectors that have particular uses for tandem mass spectrometry (Chapter 4) and studies of ion chemistry (Chapter 6). These are denoted EBE, BEB, BEE, EBEB or BEBE *etc.*

Double-focusing instruments can be scanned in a number of ways but a common scan is one in which the electric and magnetic fields are varied such that the ratio of the field strengths is always held constant (B/E = constant). These scans are known as *linked scans* that will be returned to later in Chapter 4 in the context of tandem mass spectrometry (MS/MS) experiments on magnetic sector mass spectrometers.

Double-focusing sector mass spectrometers can achieve mass resolutions up to 100,000 that allows ions which share the same nominal mass but different exact mass to be resolved. Accurate mass measurements (see Section 5.1) can be obtained where an ion's mass is measured to six decimal places (or a few parts-per-million (ppm)). This can be useful to identify the composition of an ion as discussed in Section 1.3.1.

Reverse geometry sector mass spectrometers are also useful in mass-analysed ion kinetic energy spectra (MIKES) experiments in which metastable decomposition products are detected (see Section 4.3.1).

Mass Spectrometry: A Foundation Course, K. Downard
© Royal Society of Chemistry 2004

Downloaded from http://books.rsc.org/books/monograph/chapter-pdf/1169463/bk9780854046096-00022.pdf by Ecole Polytechnique Federale de Lausanne user on 16 May 2025

3.3.3 Quadrupoles

The concept of the quadrupole mass analyser was first reported by Paul and Steinwedel in the 1950s. A quadrupole (Q) consists of four rods arranged in parallel where those opposite to one another are electrically connected (Figure 3.18). The quadrupole has a number of advantages over magnetic sector mass analysers including the low cost of construction, their compact size, and fast scanning capability.

A voltage of opposite polarity (+/−V) is applied to adjacent rods consisting of a direct current (DC) component (denoted U) and a radio-frequency (RF) (denoted $V_{RF}\cos(wt)$) component where w is the angular frequency of the RF field. Ions are accelerated out of the ion source along the z-axis between the rods. They experience forces in the x and y direction $-ze(\mathrm{d}V/\mathrm{d}x)$ and $-ze(\mathrm{d}V/\mathrm{d}y)$ that cause them to oscillate toward and away from the rods. When the oscillation becomes too large the ions strike the rods and do not reach the detector.

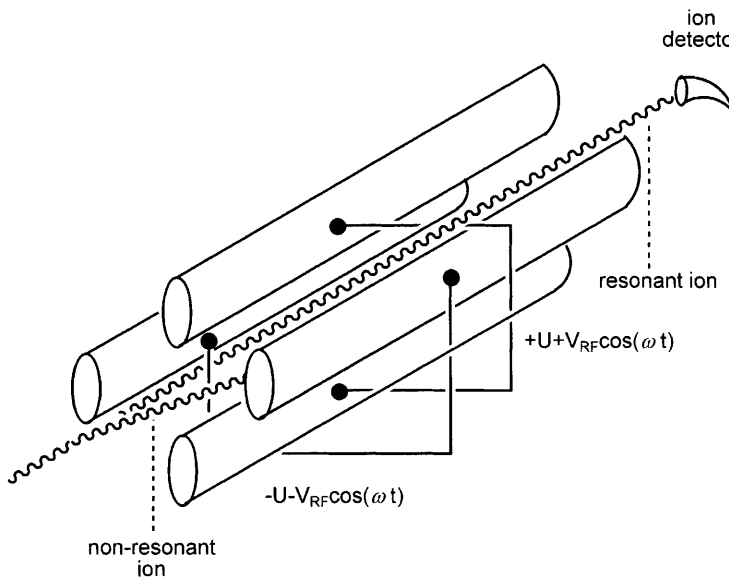

Figure 3.18 *Quadrupole mass analyser showing ion oscillation under the influence of the variable fields*

For any given analyser, the radius of an imaginary cylinder that fits in the centre of the rod (r_0) is constant as is the frequency of the RF field w. Two functions a and q define a stable trajectory for which ions

Mass Spectrometry: A Foundation Course, K. Downard
© Royal Society of Chemistry 2004

Downloaded from http://books.rsc.org/books/monograph/chapter-pdf/1169463/bk9780854046096-00022.pdf by Ecole Polytechnique Federale de Lausanne user on 16 May 2025

do not collide with the rods across a range of values for U and V_{RF} (equation 3.25 and 3.26).

$$a_z = -2a_r = -4zeU/m^2r_0^2w^2 \tag{3.25}$$

$$q_z = -2q_r = -2zeV_{RF}/m^2r_0^2w^2 \tag{3.26}$$

A plot of a versus q is known as a stability diagram where the regions below the curves for m_1 m_2 and m_3 represent the values of a and q for which ions follow a stable ion trajectory to the detector (Figure 3.19). In principle, the quadrupole can be operated over a range of values of U and V_{RF} such that the (a,q) coordinates are always below the curves. In practice, the voltages U and V_{RF} are held at a fixed ratio to maximise the mass resolution that can be achieved. This leads to an operating region defined by a line with a slope of $2U/V_{RF}$. A complete mass spectrum is obtained by scanning the voltages U and V_{RF} where this fixed ratio is maintained. Ideally the voltages U and V_{RF} should be held constant throughout the passage of ions of a particular m/z through the analyser. Scan rates of the order of 1000 u sec^{-1} are common and allow for the majority of ions of a particular m/z to reach the detector.

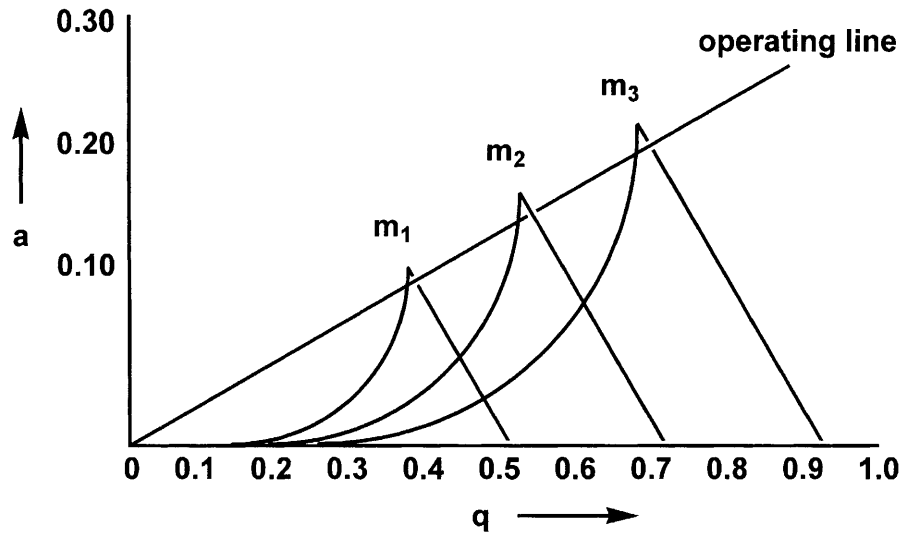

Figure 3.19 *Stability diagram indicating the values of a and q for which ions of mass m_1, m_2 and m_3 follow a stable trajectory to the ion detector*

Mass Spectrometry: A Foundation Course, K. Downard
© Royal Society of Chemistry 2004

Downloaded from http://books.rsc.org/books/monograph/chapter-pdf/1169463/bk9780854046096-00022.pdf by Ecole Polytechnique Federale de Lausanne user on 16 May 2025

Because quadrupoles operate at lower voltages and can be scanned at faster rates than magnet-based mass spectrometers, they are more easily coupled to gas and liquid chromatography instruments. However, they achieve lower mass resolutions of up to approximately 5000.

3.3.4 Quadrupole Ion Trap

There are strictly two types of ion traps though the term is usually associated with only one of them: the *quadrupole ion trap*. Quadrupole ion traps (QIT or IT) are so named because they use similar operating principles to those of the standard quadrupole mass analyser. Despite this they are constructed very differently and consist of two conical lens or electrodes, and one "donut-shaped" ring lens (Figure 3.20). Ion cyclotron resonance (ICR) mass spectrometers are also ion traps but use magnetic fields to store ions as described in the next section.

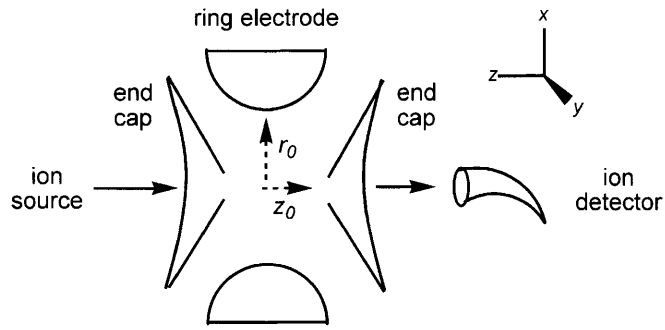

Figure 3.20 *Cross-section of a quadrupole ion trap*

In a QIT, ions are held or trapped in the small interior volume between the conical lenses that form the "caps" of the trap and the centre of the ring electrode. By lowering and raising the voltages on the entrance and exit trap electrode, ions can pass into the trap, be stored for some period of time (usually μs), and then be released to the detector. The trap is usually operated in the *mass selective stability mode* where ions of a particular *m/z* are selectively released from the trap.

Within the trap ions undergo a complex sinusoidal motion with the application of an oscillating RF potential to the ring electrode. An ion will be stored in the trap depending upon the values for the mass, m, and charge, z, of the ion, the radius of the ring electrode (r_0), the separation of the caps from the centre of the ion trap (z_0), the oscillating frequency, w, the amplitude of the potential applied to the end caps U, and the

Mass Spectrometry: A Foundation Course, K. Downard
© Royal Society of Chemistry 2004

Downloaded from http://books.rsc.org/books/monograph/chapter-pdf/1169463/bk9780854046096-00022.pdf by Ecole Polytechnique Federale de Lausanne user on 16 May 2025

amplitude of the ring electrode voltage, V_{RF}, according to equations 3.27 and 3.28.

$$a_z = -2a_r = -16zeU/m(r_0^2 + z_0^2)w^2 \quad (3.27)$$

$$q_z = -2q_r = 8zeV_{RF}/m(r_0^2 + z_0^2)w^2 \quad (3.28)$$

Again an ion stability diagram can be constructed to define the (a,q) coordinates for which ions are stored in the trap. Ions possessing values of a and q that give them both axial (along the z-axis between the caps) and radial (in the plane of the ring electrode) stability will remain trapped. In modern ion trap instruments, an inert gas such as helium (added to a pressure of about 10^{-3} Torr, or 0.1 Pa) helps to store the ions by lowering their kinetic energy through collision with the gas.

A unique feature of traps is that ions can be introduced and stored until a sufficient density is achieved for ejection and analysis. In conventional quadrupole and magnetic sector instruments, ions continuously pass through the mass spectrometer and their population is largely determined by the efficiency of the ionisation process. The ability to store ions, and the relatively short path to the detector that the ions follow in a quadrupole ion trap, can lead to very high detection sensitivities. Ion traps can also be exploited in studies of ion chemistry (see Chapter 6) by using longer containment times.

In practice, it is necessary to balance the desire to store large numbers of ions in the trap with *space charge effects* that arise from the repulsion of neighbouring ions. These repulsive forces cause ions to leave the trap and not be detected. It also perturbs the motion of ions in the trap resulting in degraded mass resolution and mass shifts that lead to large errors in mass measurements. Thus it is desirable to control the population of ions in the trap at all times during analysis. For this reason, *automatic gain control* (AGC) was developed to control the ion generation rate and couple it to the time period in which ions are introduced into the trap and stored. A typical operation of an ion trap involves lowering the end cap potential closest to the ion source to allow ions to enter the trap. This voltage is raised once sufficient numbers of ions are stored as determined by the AGC measurements. The ring electrode is held at an appropriate value to store ions over a range of m/z values. The potential on the second cap electrode is then lowered and the ring electrode voltage V_{RF} is linearly ramped to eject ions of increasing m/z in turn onto the detector.

Downloaded from http://books.rsc.org/books/monograph/chapter-pdf/1169463/bk9780854046096-00022.pdf by Ecole Polytechnique Federale de Lausanne user on 16 May 2025

Mass Spectrometry: A Foundation Course, K. Downard
© Royal Society of Chemistry 2004

3.3.5 Ion Cyclotron Resonance

Traps that make use of a magnetic field are known as *ion cyclotron resonance* (ICR) mass analysers. The name derives from the frequency of an ion's circular motion w_C within a magnetic field, B. In these instruments the trap consists of a cubic, rectangular or cylindrical "box" with entrance and exit slits cut in opposite sides or plates (Figure 3.21).

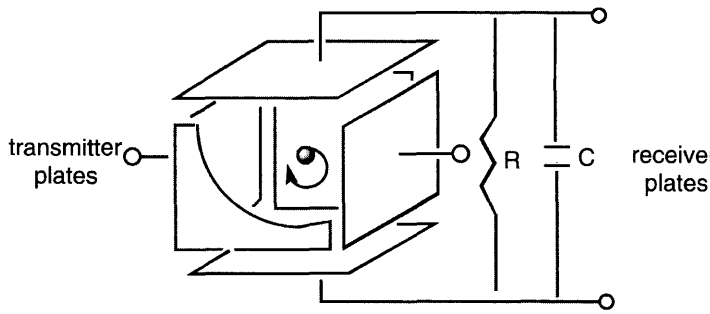

Figure 3.21 *Ion trap of an ion cyclotron resonance (ICR) mass spectrometer*

In a magnetic field, B, an ion with a velocity v will adopt a circular trajectory with a radius r perpendicular to the field when the centripetal and centrifugal forces it experiences are equal (equation 3.29).

$$zvB = (mv^2)/r \tag{3.29}$$

The angular velocity of the ion perpendicular to the field is given by equation 3.30.

$$w_C = v/r \tag{3.30}$$

Substituting equation 3.30 into equation 3.29 and simplifying, equation 3.31 is derived.

$$w_C = zB/m \tag{3.31}$$

Thus an ion's cyclotron frequency depends on its mass and charge but is independent of its velocity (equation 3.31) and the m/z ratio for an ion can be determined by measuring its cyclotron frequency. Ions of lower m/z have higher cyclotron frequencies (Figure 3.22A); ions with higher m/z have low cyclotron frequencies (Figure 3.22B). Note that oppositely charged ions would move with the same cyclotron frequency but in opposite directions were they both present in the trap.

Mass Spectrometry: A Foundation Course, K. Downard
© Royal Society of Chemistry 2004

Downloaded from http://books.rsc.org/books/monograph/chapter-pdf/1169463/bk9780854046096-00022.pdf by Ecole Polytechnique Federale de Lausanne user on 16 May 2025

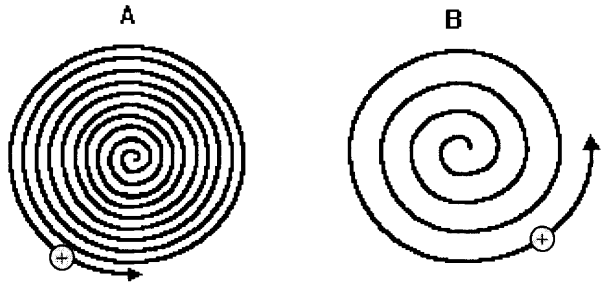

Figure 3.22 *Ion cyclotrons of high frequency or low m/z (A) and low frequency or high m/z (B)*

Ions moving in a magnetic field adopt stable cyclotron orbits and can be stored (up to several hours!) provided the pressure is kept low (typically $<10^{-8}$ Torr or 10^{-6} Pa). They do not, however, generate any detectable signal. In order to generate an electrical signal or current, ions of a particular *m/z* ratio are given extra energy through the application of an oscillating electrical field, E_t, (equation 3.32).

$$E_t = E_0 \cos w_C t \tag{3.32}$$

If, and only if, the frequency of field is the same as their cyclotron frequency, the ions absorb energy increasing their velocity and orbital radius while maintaining a constant cyclotron frequency. As these ions approach the top plate of the cell, electrons are attracted to the plate from ground. When the ions circulate towards the bottom plate, the electrons travel back down to the bottom plate. This coherent motion of ions between the two plates produces an electrical current that can be amplified and detected and is known as the *image current*. The amplitude of the current is proportional to the number of ions in the cell at this frequency. This phenomenon provides the basis for ion cyclotron resonance mass spectrometry with ions of different cyclotron frequencies unaffected.

The excitation of ions by an oscillating electric field has three main objectives in ICR mass spectrometry. First it accelerates the ions coherently to a larger orbital radius in the trap so they can be detected. Second the kinetic energy of the ions is increased to achieve their dissociation or to facilitate ion molecule or ion-ion reactions where desirable. Thirdly, it allows ions to be accelerated to radii larger than the trap to effect their removal from or collision with the trap.

In an ideal vacuum, excited ions would maintain their orbiting trajectories indefinitely. In practice, their energy is dampened and they

Mass Spectrometry: A Foundation Course, K. Downard
© Royal Society of Chemistry 2004

Downloaded from http://books.rsc.org/books/monograph/chapter-pdf/1169463/bk9780854046096-00022.pdf by Ecole Polytechnique Federale de Lausanne user on 16 May 2025

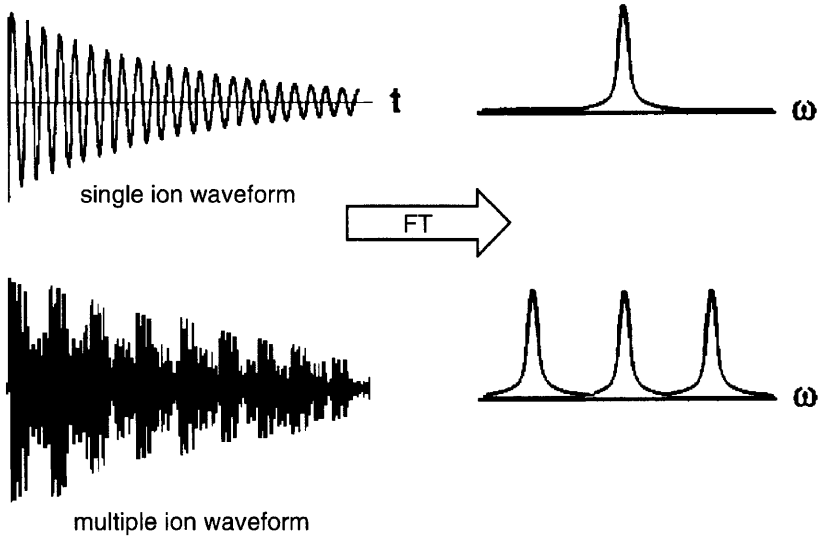

Figure 3.23 *Single and multiple ion waveforms and frequency domain spectra after application of a Fourier transform*

return to their original energy and cyclotron frequency. Therefore a plot of the amplitude of the frequency over time resembles that shown in Figure 3.23.

A complete mass spectrum is derived from the overlap of the individual ion waveforms shown in Figure 3.23. Such complicated data are best devolved through the application of a mathematical process known as a Fourier transform (FT). In a Fourier transform ion cyclotron resonance mass spectrometer, all ions in the trap are excited simultaneously to larger orbits by applying a wide range of excitation energies at once (a *broadband excitation*) in the form of an excitation *chirp*. The excitation event is very brief so that ions are not excited to orbits greater than the dimensions of the cell. Applying the Fourier transform, the combined waveform can be devolved to a frequency domain (w) spectrum from which a mass spectrum (m/z) can be assembled. As a result FT-ICR mass spectrometry (also known less descriptively as FT-MS) can detect and measures the m/z ratios of all ions in the trap simultaneously; a unique feature of this particular mass analyser.

The mass resolution ($m/\Delta m$) achieved in this type of mass analyser can be defined by equation 3.33 where Δw is the width of the peaks in the frequency domain spectrum.

$$m/\Delta m = zB/m\Delta w \tag{3.33}$$

Mass Spectrometry: A Foundation Course, K. Downard
© Royal Society of Chemistry 2004

Downloaded from http://books.rsc.org/books/monograph/chapter-pdf/1169463/bk9780854046096-00022.pdf by Ecole Polytechnique Federale de Lausanne user on 16 May 2025

Note that mass resolution increases proportionately with an increase in the magnetic field strength, B. Other performance improvements are attained by using a high magnetic field that include an increase in the number of ions that can be trapped, an increase in the maximum trapping time that is possible, and an increase in the speed of data acquistion. Therefore many modern FT-ICR mass spectrometers feature magnets that operate at 9 or even 14 Tesla. An impressive demonstration of the advanced capabilities of an FT-ICR mass spectrometer is the unit resolution of the isotope peaks for ions of large proteins, including elucidation of the protein's elemental composition after its expression in heavy isotope ^{13}C and ^{15}N-depleted media (Figure 3.24).

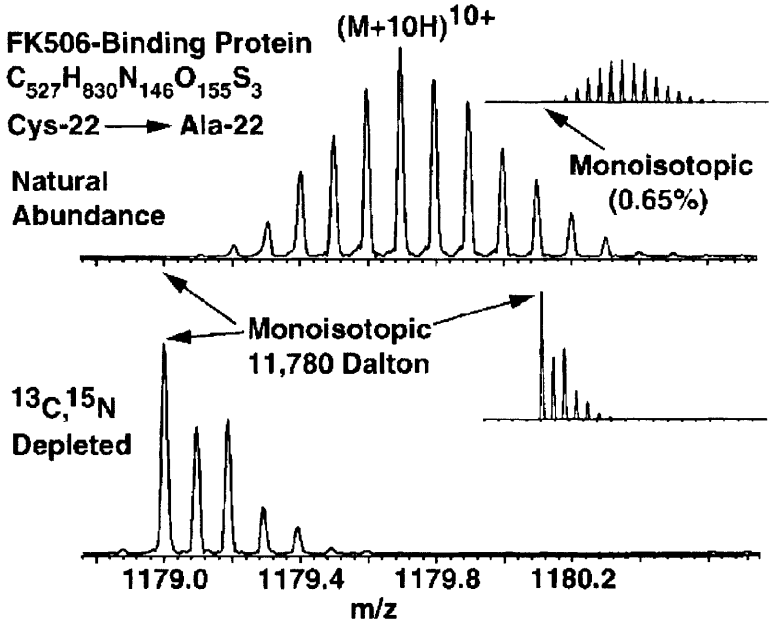

Figure 3.24 *ESI FT-ICR mass spectrum (at 9.4 Tesla) of a mutant (C22A) FK506-binding protein. Top: Natural-abundance isotopic distribution. Bottom: Isotopic distribution for the same protein grown in a medium of ^{12}C (99.95%) and ^{14}N (99.99%) together with that predicted based on chemical formula (inserts)*
(Source: A.G. Marshall, M.W. Senko, W. Li, M. Li and S. Dillon, Protein molecular weight to 1 Da by 13C, 15N double-depletion and FT-ICR mass spectrometry, *J. Am. Chem. Soc.*, 1997, **119**, 433–434)

3.3.6 Hybrid Instruments

It is often advantageous to construct mass spectrometers that consist of more than one mass analyser. Where the individual mass analysers are

Mass Spectrometry: A Foundation Course, K. Downard
© Royal Society of Chemistry 2004

Downloaded from http://books.rsc.org/books/monograph/chapter-pdf/1169463/bk9780854046096-00022.pdf by Ecole Polytechnique Federale de Lausanne user on 16 May 2025

of a different type (TOF, magnetic sector, quadrupole, ion trap or ICR) the combined mass spectrometer is known as a *hybrid* instrument. Since different mass analysers offer complementary features in terms of mass resolving power, mass range and ion transmission, a combination of different mass analysers in a single instrument can lead to performance enhancements and/or benefits for certain experiments, particularly tandem mass spectrometry described in Chapter 4.

In principle, any combination of two or more *different* mass analysers produces a hybrid instrument but in practice some are more suitable for coupling than others. A few hybrid instruments in common use today are those featuring combinations of magnetic sectors and quadrupoles (such as the configuration BE-Q, consisting of a magnet, electric sector and quadrupole), and a quadrupole and time-of-flight analyser (Q-TOF).

A comparison of the performances of the various mass analysers is represented in Appendix 4. Now that the formation and separation of ions within a mass spectrometer has been considered, it is time to understand in some detail how they are detected.

3.4 DETECTORS

Once ions leave the mass analyser they pass to the ion detector (except in the case of the ICR) where they generate an image current. The earliest mass spectrometers detected ions by means of photographic paper onto which line images were visualised. Many different types of detectors are now in use, the choice of which depends in part on the nature of the mass analyser. To begin, the simplest detector used in mass spectrometry, the so-called *Faraday cup or cage* will be considered.

3.4.1 Faraday Cup

The Faraday cup or cage consists of a long thin rectangular box arranged such that the incoming ion beam strikes the base of the box at the *collector*. The collector plate is usually angled steeply with respect to the ion beam in order to prevent reflected ions and secondary electrons emitted from the plate from escaping. Each collector box is typically enclosed by a second box (held at ground) that serves to shield the detector from electrical noise. A slot on the front of this box acts as a resolving slit. When positive ions strike the collector plate they are neutralised by electrons drawn from ground across a resistor. This current is amplified and recorded. Faraday cup detectors are extremely simple in their design and are an inexpensive and reliable alternative to other detectors. They are best used for quantitation and accurate mass

Mass Spectrometry: A Foundation Course, K. Downard
© Royal Society of Chemistry 2004

Downloaded from http://books.rsc.org/books/monograph/chapter-pdf/1169463/bk9780854046096-00022.pdf by Ecole Polytechnique Federale de Lausanne user on 16 May 2025

measurements where ion currents do not change appreciably during the course of a measurement. The smallest ion current that can be detected is of the order of 10^{-15} A. Faraday cups are less suited to experiments where the ion current changes quickly, as is the case in experiments conducted with online chromatography where the mass analyser is rapidly scanned and the population of ions transmitted to the detector varies.

3.4.2 Electron Multipliers

Electron multipliers (EM) achieve higher detection efficiencies than Faraday cups by amplifying the secondary electrons emitted from the detector surface. There are two common types; one in which the detector surface is constructed of a series of discrete plates (*discrete dynode*) connected by chain of resistors, and a second in which a single, continuous detector surface is used (*continuous dynode*) (Figure 3.25).

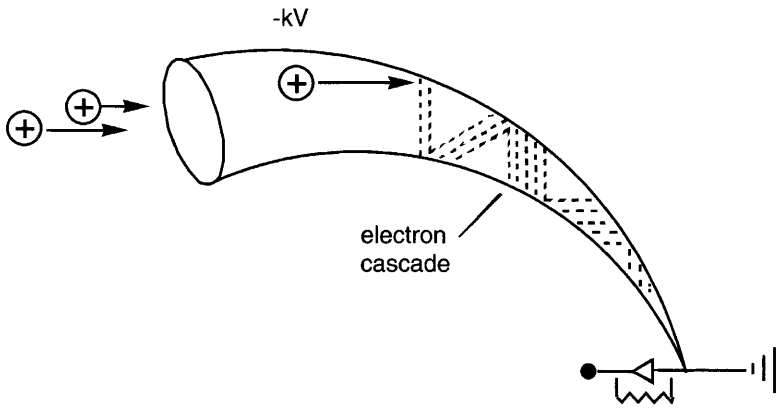

Figure 3.25 *Schematic representation of a continuous dynode electron multiplier*

In the discrete dynode detector, a high voltage applied across the resistor chain leads to an equal voltage difference between each successive plate surface. The plates are constructed principally of a beryllium/copper alloy or aluminium onto which oxides of beryllium or aluminium coat the surface. The multiplier must not be exposed to air when operational as this can damage the coatings and prevent its further use. The multiplier is assembled such that the dynodes have progressively higher voltages from the first to the last (over some 10–20 plates). As ions strike the first plate, secondary electrons are emitted from the surface and projected to the second dynode plate. This process repeats itself leading to a *cascading effect*. The amplified current (with gains of typically 10^7 to 10^8 during the detector's useful life of operation) is finally detected. In

Mass Spectrometry: A Foundation Course, K. Downard
© Royal Society of Chemistry 2004

Downloaded from http://books.rsc.org/books/monograph/chapter-pdf/1169463/bk9780854046096-00022.pdf by Ecole Polytechnique Federale de Lausanne user on 16 May 2025

the alternate continuous dynode electron multiplier, the internal surface acts as a resistor so that an electrical gradient is achieved across the detector. The secondary electrons emitted from the collision of ions with the detector surface are accelerated to the tip of the detector amplifying the current.

Current gains of the order of 10^5 are typically achieved after which the detector response plateaus. Electron multipliers are commonly used in conjunction with scanning quadrupole and magnetic sector mass analysers. A disadvantage of these detectors is that the emission of secondary electrons is dependent on the mass and charge of the incident ions. That is, ions with a *m/z* ratio of 500 will produce a different detector response than ions with a *m/z* of 5000. This non-linear detector response leads to difficulties in quantitation experiments, where the ion signal is used to reflect the levels of each component in a sample mixture.

3.4.3 Microchannel Plate Electron Multipliers

Microchannel plate (MCP) detectors consist of a thin flat plate containing series of small (~10 μm) channels (Figure 3.26). The plates that differentiate the channels are typically angled at several degrees to the incident ion beam. Ions pass into any of the channels, strike the plate walls and emit electrons that are deflected to the opposite plate. This process repeats itself thereby amplifying the electron current. Gains of the order of 10^5 can be achieved with one plate. Amplification can reach 10^8 using a stack of several plates.

These devices are commonly used in TOF mass analysers since ions are unfocused and arrive at the detector over large areas. They also are implemented in array detectors.

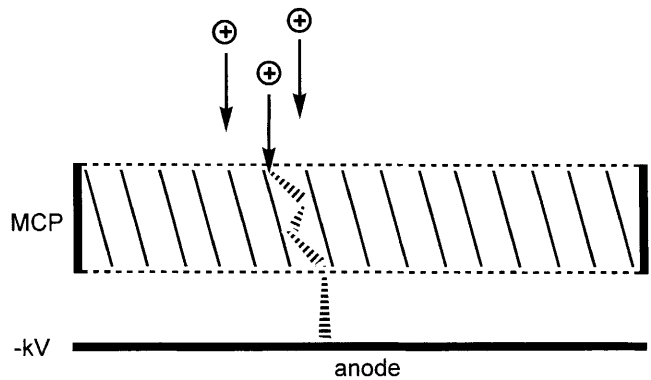

Figure 3.26 *Schematic representation of a micro-channel plate (MCP) detector*

Mass Spectrometry: A Foundation Course, K. Downard
© Royal Society of Chemistry 2004

Downloaded from http://books.rsc.org/books/monograph/chapter-pdf/1169463/bk9780854046096-00022.pdf by Ecole Polytechnique Federale de Lausanne user on 16 May 2025

3.4.5 Array Detectors

A limitation of scanning mass analysers (quadrupoles and magnetic sectors) over non-scanning TOF instruments, is that only ions of a particular *m/z* ratio are detected at any particular point in time. This compromises sensitivity since many ions passing into the mass spectrometer are actually deflected from the detector and go undetected.

Beginning in the mid 1970s, array detectors were constructed for magnetic sector mass spectrometers that enabled all ions, or ions over a large range of *m/z* ratios, to be detected simultaneously.

Photodiode arrays (PDA) are the most common form in which a microchannel plate detector is coupled to fibre optical channels *via* a phosphorescent screen (Figure 3.27).

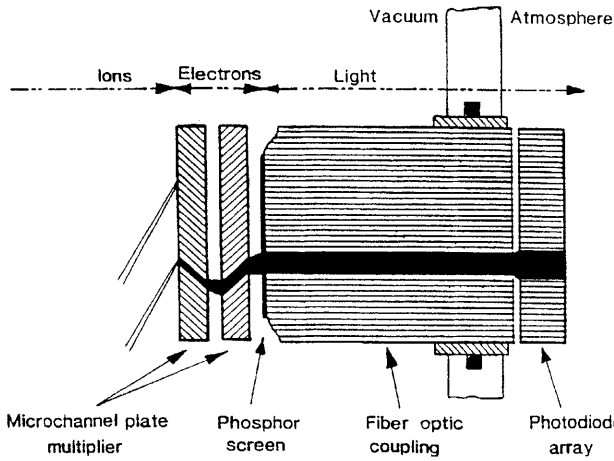

Figure 3.27 *Schematic representation of a photodiode array (PDA) detector* (Source: S. Evans, in *Methods Enzymology*, McCloskey (ed), Academic Press, New York, 1990, Vol. 193, Ch. 3, p. 80, Figure 9)

Ions enter the microchannel plates and the electrons emitted strike the phosphorescent screen. The screen consists of a layer of aluminium coated with crystalline phosphor often composed of caesium iodide (CsI) and thallium (Tl). This surface emits light in the form of photons that are transmitted down the fiber optic channels onto a charge or plasma-coupled device (CCD or PCD). The photodiodes of the CCD or PCD, unlike the phosphor screen of the image intensifier, react independently of each other. Each pixel of the photodiode array is typically 25 μm by 2.5 mm in size and converts the photons to charge. The photodiodes of the CCD array record the charge necessary

Mass Spectrometry: A Foundation Course, K. Downard
© Royal Society of Chemistry 2004

Downloaded from http://books.rsc.org/books/monograph/chapter-pdf/1169463/bk9780854046096-00022.pdf by Ecole Polytechnique Federale de Lausanne user on 16 May 2025

to neutralise that accumulated in each pixel and this data is integrated and stored.

Array detectors constructed for magnetic sector mass spectrometers are able to detect ions across some 5% of the full *m/z* range of the instrument. Detection ranges of approximately 25% are possible for quadrupole instruments albeit with some mass discrimination; that is, the preferential detection of ions at one end of the range over the other.

Other array detectors, such as the position and time-resolved ion collector (PATRIC) have been developed for commercial use. Like the photodiode array, ions first strike a microchannel plate. The electrons emitted from the MCP are accelerated to a collector stack consisting of a series (some 50) of conductive strips connected together by a capacitor chain. Charge amplifiers located at each end of the stack detect the current passing through the capacitors. The time the ions take to pass through the array depends on their mass and charge so that ions of a particular *m/z* value can be tracked as they pass across the array.

The performance of this array is similar to a PDA. All array detectors result in some compromise in mass resolution since even a focused ion beam can pass through the array at multiple positions or channels. Unit resolution across most of the mass range can usually be obtained, although at some expense in terms of sensitivity.

3.5 COMPUTER ACQUISITION OF DATA

3.5.1 Role of Computers in Mass Spectrometry

Computers now play a central role in all mass spectrometry experiments. Computers are used for instrument control, data acquisition, data processing and storage. Once the sample is introduced into the mass spectrometer, all remaining functions are performed under computer-control. This includes mass calibration and tuning of the instrument to optimise the ion signal at the detector.

Both personal computers and UNIX-based workstations are used to control mass spectrometers. Mass spectrometry experiments are pre-programmed using the computer software where method files control instrument parameters including source temperatures, gas pressures, lens and detector voltages. The same computer is typically also used to control peripheral devices such as gas and high pressure liquid chromatographs when these are coupled to the mass spectrometer. Computers allow the chromatographic conditions to be pre-programmed and the user to monitor the results at a second detector (such as a UV

Mass Spectrometry: A Foundation Course, K. Downard
© Royal Society of Chemistry 2004

Downloaded from http://books.rsc.org/books/monograph/chapter-pdf/1169463/bk9780854046096-00022.pdf by Ecole Polytechnique Federale de Lausanne user on 16 May 2025

detector) in addition to the mass spectral data within a common interface.

Computers also allow a multitude of different scan experiments to be performed during a single acquisition. Tandem mass spectra (see Chapter 4) can be recorded where sufficient ion current for a nominated *m/z* is detected in a preliminary MS scan. This enables both molecular weight and structural information to be derived in an automated manner.

3.5.2 Analog-to-Digital Converters

Since the mass spectrometer acquires data in an analog format, an *analog-to-digital converter* (ADC) is used to convert the detector response into a digital format. The ion current read by the detector is amplified and filtered to remove high frequency noise. This current, recorded over the time period of the experiment, is plotted on a mass-to-charge scale by comparison with data obtained from an earlier mass calibration experiment. In a mass calibration experiment, ion signals produced from a well-characterised sample or sample mixture are assigned to their theoretical mass values. This is often performed under computer control with reference to a table of stored theoretical masses.

3.5.3 Data Processing and Interpretation Algorithms

Data interpretation and processing are also significantly aided by the use of computers. Sections of a mass spectrum can be replotted, normalised (where the largest peak is plotted to the 100% level on the *y*-axis), and "smoothed". Smoothing algorithms are applied to filter extraneous noise that appears in a mass spectrum due to electronic interference and background ion current from ions associated with the analyte. A subtraction algorithm can also be applied to remove such signals, where mass spectra representative of the background are pre-recorded independent of the sample ions.

Where the ion current is monitored during multiple scans of the mass analyser over the course of the experiment, an ion chromatogram is also recorded. Processing of this data set enables a mass spectrum to be generated from any one scan or the sum of several scans. A selected ion chromatogram can also be generated from the data set by extracting all time points along the chromatogram during which ions of a particular *m/z* were detected. This is useful when a sample is first subjected to chromatographic or electrophoretic separation prior to mass spectrometric analysis. Components of a sample mixture will subsequently only

Downloaded from http://books.rsc.org/books/monograph/chapter-pdf/1169463/bk9780854046096-00022.pdf by Ecole Polytechnique Federale de Lausanne user on 16 May 2025

Mass Spectrometry: A Foundation Course, K. Downard
© Royal Society of Chemistry 2004

pass into the mass spectrometer over a limited period of time subject to the performance of the experiment. In other words, a component that elutes early from a chromatographic or electrophoretic column will appear in mass spectra recorded early in the experiment, but not in spectra recorded later. The converse is also true. Thus the time at which a component is introduced and detected within the mass spectrometer can be obtained from the ion chromatogram.

In addition to data acquisition, analysis and processing, many other programs have been written to assist with data interpretation. These include those that deduce the composition of the ion based upon its isotopic distribution, the conversion of *m/z* ratios for multiply-charged ions into a molecular weight value, the structure of an analyte based on tandem mass spectral data (see Chapter 4), and identification of an analyte by comparison of a mass spectrum with data stored in a library or database. The importance of mass spectral libraries and the use of such databases will be discussed in later chapters (Chapters 5 and 8).

3.6 VACUUM PUMPS

An important component of all mass spectrometers, not often considered during operation, are the vacuum pumps. Many instruments use at least two pumps; one to evacuate the instrument chamber of air after assembly or venting and a second to reduce the pressure to that required to operate. Pressures vary within different mass spectrometers, and within each component, but it is typical for instruments to operate over a range of 10^{-3} to 10^{-6} Pa (1 Pa = 133.3226 torr or millimetres of mercury, mmHg). As described in Section 3.1, mass spectrometers operate under vacuum to prevent the collision and reaction of ions with residual gas molecules during their flight from the ion source to the detector. Low pressures also prevent condensation from building up inside the instrument that would coat critical lens and surfaces and create electrical discharges. The detector, too, must be protected from moisture and oxidation for it to operate properly.

3.6.1 Rotary Pumps

The most efficient way in which to reduce the pressure in a mass spectrometer to the 1 Pa level is with a *rotary* or *roughing pump*. In this pump, a rotating inner barrel draws the gas in from one side of a chamber and compresses it on the other (Figure 3.28). The compressed gas passes through a hydrocarbon oil reservoir, where the vapour pressure of the oil

Mass Spectrometry: A Foundation Course, K. Downard
© Royal Society of Chemistry 2004

Downloaded from http://books.rsc.org/books/monograph/chapter-pdf/1169463/bk9780854046096-00022.pdf by Ecole Polytechnique Federale de Lausanne user on 16 May 2025

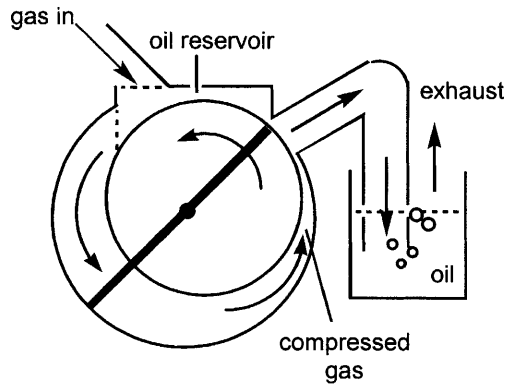

Figure 3.28 *Cross-section of a rotary vacuum pump*

influences the lowest pressure that can be obtained. Rotary pumps have the capacity to dispel approximately 100 litres of gas per minute through the pump.

3.6.2 Diffusion Pumps

Diffusion pumps are used in conjunction with a roughing or rotary pump and operate at pressures below 1 Pa. Unlike rotary pumps, diffusion pumps use no moving parts and operate by expelling the vapour trapped in a high-boiling oil reservoir. Vapours pass up a chimney, cool and

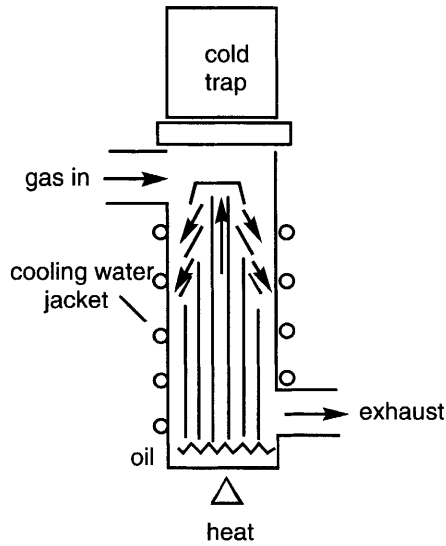

Figure 3.29 *Cross-section of a diffusion pump*

Mass Spectrometry: A Foundation Course, K. Downard
© Royal Society of Chemistry 2004

Downloaded from http://books.rsc.org/books/monograph/chapter-pdf/1169463/bk9780854046096-00022.pdf by Ecole Polytechnique Federale de Lausanne user on 16 May 2025

saturate the gas molecules in the pump dragging them to the base. This compresses the gas in the pump. The oil vapour is continually recycled to achieve pressures of the order of 10^{-6} Pa (Figure 3.29).

A serious problem with diffusion pumps is that should the instrument become vented while in operation, oil would be dragged from the reservoir into the spectrometer. A cold trap is generally positioned at the head of the pump to avoid oil and vapour from entering the mass spectrometer. Preventing the oil from reaching a high temperature is also necessary to avoid problems. This is achieved by placing a water-cooled jacket around the pump and through the application of synthetic polyphenyl and silicone lubricants that can better withstand continual heating and cooling.

3.6.3 Turbomolecular Pumps

A popular high vacuum pump is a *turbomolecular pump* often known simply as a *turbo*. Turbos are constructed of a turbine compressor, like that used in a jet engine, and are often preferred over a diffusion pump for several reasons. First, the pumps use no oil exposed to vacuum. Second, the pump can attain full operation more quickly than a diffusion pump leading to faster evacuation times whenever a mass spectrometer is opened for repair or service.

Within a turbo pump, a fast moving rotor or set of rotors (operating at typically 50,000 revolutions per minute) draws gas through the pump at a capacity of the order of 10–100 l s^{-1} (Figure 3.30). Because of the speed of the rotors, the pump must be machined to low tolerances in order for it to operate efficiently for several years. The gas is further passed

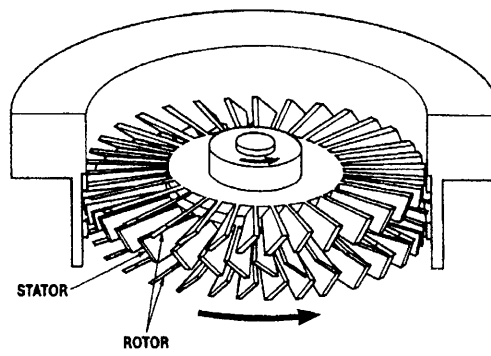

Figure 3.30 *View of the rotor of a turbomolecular pump*
(Source: J.T. Watson, *Introduction to Mass Spectrometry*, 3rd edition, Lippincott-Raven, Philadelphia, 1997, Figure 19.7, p. 431)

Mass Spectrometry: A Foundation Course, K. Downard
© Royal Society of Chemistry 2004

Downloaded from http://books.rsc.org/books/monograph/chapter-pdf/1169463/bk9780854046096-00022.pdf by Ecole Polytechnique Federale de Lausanne user on 16 May 2025

through a roughing pump to the exhaust. It is important that the pump is vented to atmospheric pressure before it is stopped to prevent oil being dragged from the rotary backing pump into the turbo. Pressures of the order of 10^{-6} to 10^{-7} Pa can be obtained. Turbomolecular pumps are commonly used in quadrupole and magnetic sector mass spectrometers. In the case of the latter, multiple turbo pumps flank regions of the flight tube to maintain suitable operating pressures throughout.

3.6.4 Cryopumps

Among the pumps that offer the lowest pressure are *cryogenic pumps*. Cryogenic pumps achieve pressures as low as 10^{-9} Pa by condensing residual gases on surfaces maintained at extremely low temperature. These coldheads operate at about 20 K (–278 °C) by means of liquid helium. The low temperatures are maintained by expanding the helium gas held at high pressure. As helium passes through heat exchangers (made of a fine metal mesh with a high heat capacity) on its way back to the refrigeration compressor, heat is removed thereby cooling the gas. A typical design construction is shown in Figure 3.31.

Cryopumps create a vacuum by condensing and freezing most of the gases in the vacuum chamber on several helium-cooled coldheads. The

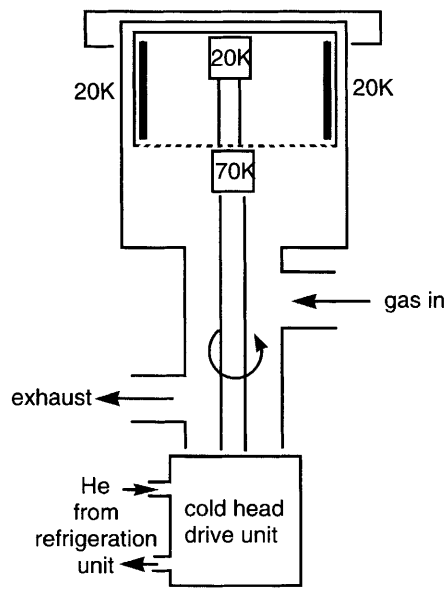

Figure 3.31 *Cross-section of a cryopump*

Mass Spectrometry: A Foundation Course, K. Downard
© Royal Society of Chemistry 2004

Downloaded from http://books.rsc.org/books/monograph/chapter-pdf/1169463/bk9780854046096-00022.pdf by Ecole Polytechnique Federale de Lausanne user on 16 May 2025

process used is the same familiar process that causes water vapour to condense on a bathroom mirror.

The cryopump condenses water vapour in the pump's first stage. The first stage operates at a temperature of 70 K (–228 °C). Most gases in air (O_2, N_2, *etc.*) in the vacuum chamber are frozen onto a second-stage condensing array by an identical process. The second stage operates at a temperature of around 20 K (–278 °C). Since the gases hydrogen, helium, and neon cannot be frozen at these temperatures a portion of the second stage contains a surface coated with highly porous activated charcoal. These gases are absorbed onto the charcoal and removed from the vacuum chamber. As the charcoal becomes saturated with gas, however, the pump's ability to efficiently maintain low pressures begins to deteriorate. To overcome this, after operation for a day or two, the cryopumps of a mass spectrometer are vented and the coldheads warmed to release the condensed and absorbed gases.

Cryopumps are commonly used today in FT-ICR mass spectrometers where very low pressures are required during ion detection periods to minimise ion-molecule and ion-ion interactions that can dampen an ion's coherent motion. This allows the characteristic high mass resolutions of these analysers to be achieved.

FURTHER READING

J.T. Watson, *Introduction to Mass Spectrometry*, 3rd edition, Lippincott-Raven, Philadelphia, 1997

E. DeHoffmann and J. Charette, *Mass Spectrometry – Principles and Applications*, 2nd edition, John Wiley & Sons, New York, 2001.

A.G. Harrison, *Chemical Ionization Mass Spectrometry*, 2nd edition, CRC Press, Boca Raton, Florida, 1992.

H.D. Beckey, *Principles of Field Ionization and Field Desorption Ionization in Mass Spectrometry*, Pergamon Press, Oxford, 1977.

R.D. Macfarlane and D.F. Torgerson, Californium-252-plasma desorption time-of-flight mass spectrometry, *Int. J. Mass Spectrom. Ion Phys.* 1976, **21(1–2)**, 81–92.

M. Barber, R.S. Bordoli, R.D. Sedgwick and A.N. Tyler, Fast atom bombardment of solids (FAB): a new ion source for mass spectrometry, *J. Chem. Soc. Chem. Commun.*, 1981, 325–327.

K. Tanaka, H. Waki, Y. Ido, S. Akita, Y. Yoshida and T. Yoshida, Protein and polymer analyses up to m/z 100,000 by laser ionization time-of-flight mass spectrometry, *Rapid Comm. Mass Spectrom.*, 1988, **2**, 151.

Mass Spectrometry: A Foundation Course, K. Downard
© Royal Society of Chemistry 2004

Downloaded from http://books.rsc.org/books/monograph/chapter-pdf/1169463/bk9780854046096-00022.pdf by Ecole Polytechnique Federale de Lausanne user on 16 May 2025

M. Karas and F. Hillenkamp, Laser desorption ionization of proteins with molecular masses exceeding 10,000 daltons, *Anal. Chem.*, 1988, **60(20)**, 2299–2301.

C.R. Blakley, M.L. Vestal, Thermospray interface for liquid chromatography/mass spectrometry, *Anal. Chem.*, 1983, **55(4)**, 750–754.

M. Yamashita and J.B. Fenn, Electrospray ion source. Another variation on the free-jet theme, *J. Phys. Chem.*, 1984, **88(20)**, 4451–4459.

R. Cotter *Time-of-Flight Mass Spectrometry. Instrumentation and Applications in Biological Research*, American Chemical Society, 1997.

W. Paul, H. Steinwedel, A new mass spectrometer without magnetic field, *Z. Naturforsch.*, 1953, **8A**, 448–450.

R.E. March, R.J. Hughes, *Quadrupole Storage Mass Spectrometry*, Wiley-Interscience, 1989.

R.E. March and J.F.J. Todd (eds) *Practical Aspects of Ion Trap Mass Spectrometry*, CRC Press, 1996.

M. Oehme, *Practical Introduction to GC-MS Analysis with Quadrupoles*, John Wiley & Sons, 1999.

A.G. Marshall, C.L. Hendrickson and G.S. Jackson, Fourier transform ion cyclotron resonance mass spectrometry: A primer, *Mass Spectrom. Rev.*, 1998, **17**, 1–35.

A.G. Marshall and F.R. Verdun, *Fourier Transforms in NMR, Optical, and Mass Spectrometry: A User's Handbook*, Elsevier, Amsterdam, 1990.

J.R. Chapman, *Computers in Mass Spectrometry*, Academic Press, New York, 1978.

Downloaded from http://books.rsc.org/books/monograph/chapter-pdf/1169463/bk9780854046096-00022.pdf by Ecole Polytechnique Federale de Lausanne user on 16 May 2025

Mass Spectrometry: A Foundation Course, K. Downard
© Royal Society of Chemistry 2004