extraction lenses. The ions collide with residual gas molecules, increase their internal energy, which induces their final desolvation, and the ion clusters disappear. However, these collisions can also give enough energy to induce ion fragmentation. This kind of fragmentation is called in-source fragmentation. The desolvation should be maximized to get a gain in sensitivity because the distribution of an analyte in different species, which are clusters, leads to a decrease in detection sensitivity. Furthermore, the formation of clusters should be controlled when doing quantitative analysis because the analyte of interest should be in a well-defined and stable form.

The transfer of ions from atmospheric pressure to the vacuum of a spectrometer necessarily induces ion losses. But these losses are compensated by the higher total ion yield in the API source due to fast thermal stabilization at atmospheric conditions. Indeed, when the sample ionization is performed under atmospheric pressure [50,51], an ionization efficiency 10^3 to 10^4 times as great as in a reduced-pressure CI source is obtained.

The early API source designs used an axial configuration. The ions were produced in the axis of the orifice. Designs have changed, however. Now the orthogonal configuration to introduce the ions into the interface is used in many API sources. The main advantage is that the orifice is no longer saturated by solvent. Instead, only ions are directed towards the inlet. Consequently, orifices can be larger than in the axial configuration. The combination of larger orifices and noise reduction largely compensates for transmission losses due to the orthogonal geometry, giving a large gain in sensitivity. Another advantage of this configuration is that the flow rates can be increased. Furthermore, this configuration gives better protection of the orifice against contamination or clogging, giving a gain in robustness. However, the orthogonal configuration with indirect trajectory of analyte ions also introduces unwanted discrimination based on mass or charge.

The most important advantage of API sources is the simplicity for the direct on-line coupling of separation techniques (HPLC, CE, etc.) to the mass spectrometer. Another attractive aspect of these sources is the easy introduction of the sample into a mass spectrometer because the operation at atmospheric pressure outside of the mass spectrometer eliminates the complicated procedure of introducing the sample into its high vacuum.

1.11 Electrospray

In the literature, electrospray is abbreviated to either ESI or ES. Because ES is ambiguous, we prefer to use ESI. The success of ESI started when Fenn *et al.* [52,53] showed that multiply charged ions were obtained from proteins, allowing their molecular weight to be determined with instruments whose mass range is limited to as low as 2000 Th. At the beginning, ESI was considered as an ionization source dedicated to protein analysis. Later on, its use was extended not only to other polymers and biopolymers, but also to the analysis of small polar molecules. It appeared, indeed, that ESI allows very high sensitivity to be reached and is easy to couple to high-performance liquid chromatography HPLC, μHPLC or capillary electrophoresis. ESI principles and biological applications have been extensively reviewed [54–56]. Several edited books on this subject also appeared in 1996 and 1997 [57,58].

ESI [59–64] is produced by applying a strong electric field, under atmospheric pressure, to a liquid passing through a capillary tube with a weak flux (normally 1–10 $\mu l\,min^{-1}$). The electric field is obtained by applying a potential difference of 3–6 kV between this capillary and the counter-electrode, separated by 0.3–2 cm, producing electric fields of the

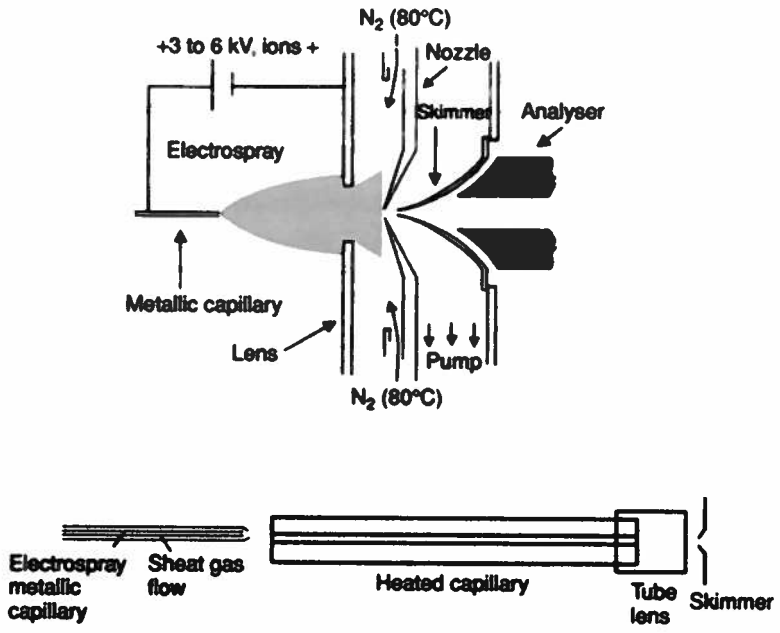

Figure 1.20
Diagram of electrospray sources, using skimmers for ion focalization and a curtain of heated nitrogen gas for desolvation (top), or with a heated capillary for desolvation (bottom).

order of 10^6 V m^{-1} (Figure 1.20). This field induces a charge accumulation at the liquid surface located at the end of the capillary, which will break to form highly charged droplets. A gas injected coaxially at a low flow rate allows the dispersion of the spray to be limited in space. These droplets then pass either through a curtain of heated inert gas, most often nitrogen, or through a heated capillary to remove the last solvent molecules.

The spray starts at an 'onset voltage' that, for a given source, depends on the surface tension of the solvent. In a source which has an onset voltage of 4 kV for water (surface tension 0.073 N m^{-2}), 2.2 kV is estimated for methanol (0.023 N m^{-2}), 2.5 kV for acetonitrile (0.030 N m^{-2}) and 3 kV for dimethylsulfoxide (0.043 N m^{-2}) [65]. If one examines with a microscope the nascent drop forming at the tip of the capillary while increasing the voltage, as schematically displayed in Figure 1.21, at low voltages the drop appears spherical, then elongates under the pressure of the accumulated charges at the tip in the stronger electric field; when the surface tension is broken, the shape of the drop changes to a 'Taylor cone' and the spray appears.

Gomez and Tang [66] were able to obtain photographs of droplets formed and dividing in an ESI source. A drawing of a decomposing droplet is displayed in Figure 1.22. From their observations, they concluded that breakdown of the droplets can occur before the limit given by the Rayleigh equation is reached because the droplets are mechanically deformed, thus reducing the repulsion necessary to break down the droplets.

The solvent contained in the droplets evaporates, which causes them to shrink and their charge per unit volume to increase. Under the influence of the strong electric field,

Figure 1.21
Effect of electrospray potential on the drop at the tip of the capillary, as observed with binoculars while increasing the voltage. Left: at low voltage, the drop is almost spherical. Centre: at about 1 or 2 kilovolts, but below the onset potential, the drop elongates under the pressure of the charges accumulating at the tip. Right: at onset voltage, the pressure is higher than the surface tension, the shape of the drop changes at once to a Taylor cone and small droplets are released. The droplets divide and explode, producing the spray.

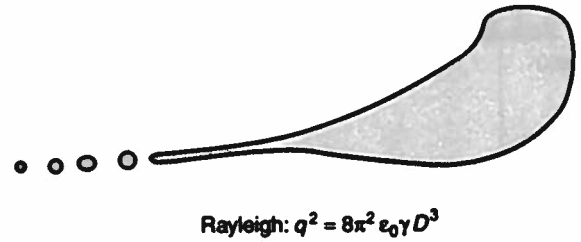

Figure 1.22
A decomposing droplet in an electrospray source, according to [66]; q, charge; ε_0, permittivity of the environment; γ, surface tension and D, diameter of a supposed spherical droplet.

deformation of the droplet occurs. The droplet elongates under the force resulting from the accumulation of charge, similarly to what occurred at the probe tip, and finally produces a new Taylor cone. From this Taylor cone, about 20 smaller droplets are released. Typically a first-generation droplet from the capillary will have a diameter of about 1.5 μm and will carry around 50 000 elementary charges, or about 10^{-14} C. The offspring droplets will have a diameter of 0.1 μm and will carry 300 to 400 elementary charges. The total volume of the offspring droplets is about 2 % of the precursor droplet but contain 15 % of the charge. The charge per unit volume is thus multiplied by a factor of seven. The precursor droplet will shrink further by solvent evaporation and will produce other generations of offspring.

These small, highly charged droplets will continue to lose solvent, and when the electric field on their surface becomes large enough, desorption of ions from the surface occurs [65]. Charges in excess accumulate at the surface of the droplet. In the bulk, analytes as well as electrolytes whose positive and negative charges are equal in number are present at a somewhat higher concentration than in the precursor droplet. The desorption of charged molecules occurs from the surface. This means that sensitivity is higher for compounds whose concentration at the surface is higher, thus more lipophilic ones. When mixtures of compounds are analysed, those present at the surface of droplets can mask, even completely, the presence of compounds which are more soluble in the bulk. When the droplet contains very large molecules, like proteins for example, the molecules will not desorb, but are freed by evaporation of the solvent. This seems to occur when the molecular weight of the compounds exceeds 5000 to 10 000 Da.

The ions obtained from large molecules carry a greater number of charges if several ionizable sites are present. Typically, a protein will carry one charge per thousand daltons

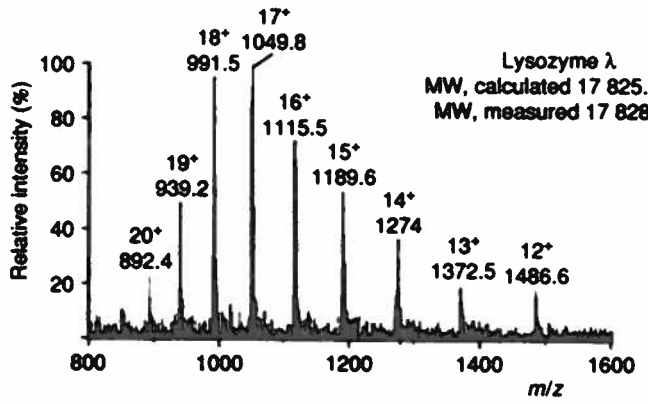

Figure 1.23
ESI spectrum of phage λ lysozyme; *m/z* in Th and the number of charges are indicated on each peak. The molecular mass is measured as being 17 828 ± 2.0 Da.

approximately, less if there are very few basic amino acids. As an example, the ESI spectrum of phage lambda lysozyme is shown in Figure 1.23. Small molecules, say less than a thousand daltons, will produce mainly monocharged ions. ESI can also be used in the case of molecules without any ionizable site through the formation of sodium, potassium, ammonium, chloride, acetate or other adducts.

ESI has important characteristics: for instance, it is able to produce multiply charged ions from large molecules. The formation of ions is a result of the electrochemical process and of the accumulation of charge in the droplets. The ESI current is limited by the electrochemical process that occurs at the probe tip and is sensitive to concentration rather than to total amount of sample.

1.11.1 Multiply Charged Ions

Large molecules with several ionizable sites produce by ESI multiply charged ions, as shown for lysozyme positive ions in Figure 1.23.

Obtaining multiply charged ions is advantageous as it improves the sensitivity at the detector and it allows the analysis of high-molecular-weight molecules using analysers with a weak nominal mass limit. Indeed, the technical characteristics of mass spectrometers are such that the value being measured is not the mass, but the mass-to-charge ratio *m/z*.

The ESI mass spectra of biological macromolecules normally correspond to a statistical distribution of consecutive peaks characteristic of multiply charged molecular ions obtained through protonation $(M + zH)^{z+}$, or deprotonation $(M - zH)^{z-}$, with minor if any contributions of ions produced by dissociations or fragmentations. However, as the measured apparent mass is actually *m/z*, to know *m* one needs to determine the number of charges *z*.

Consider a positive ion with charge z_1 whose mass-to-charge ratio is measured as being m_1 Th, issued from a molecular ion with mass *M* Da to which z_1 protons have been added. We then have

$$z_1 m_1 = M + z_1 m_p$$

where m_p is the mass of the proton.

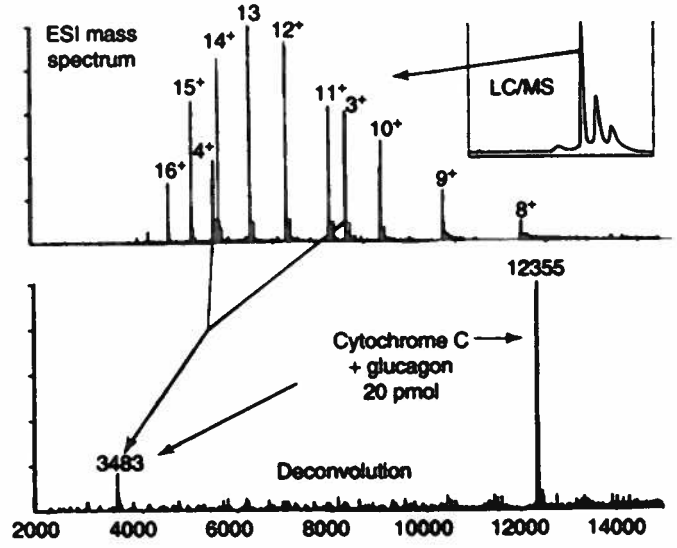

Figure 1.24
Deconvolution of an ESI spectrum of a protein mixture. From Finnigan documentation. Reprinted, with permission.

An ion separated from the first one by $(j - 1)$ peaks, in increasing order of mass-to-charge ratio, has a measured ratio of m_2 Th and a number of charges $z_1 - j$, so that

$$m_2(z_1 - j) = M + (z_1 - j)m_p$$

These two equations lead to

$$z_1 = \frac{j(m_2 - m_p)}{(m_2 - m_1)} \quad \text{and} \quad M = z_1(m_1 - m_p)$$

In the case of negative multiply charged ions, analogous equations lead to

$$z_1 = \frac{j(m_2 + m_p)}{(m_2 - m_1)} \quad \text{and} \quad M = z_1(m_1 + m_p)$$

In the example shown in Figure 1.23 using the peaks at *m/z* 939.2 and 1372.5 ($j = 6$), we obtain $z_1 = 6(1372.5 - 1.0073)/(1372.5 - 939.2) = 19$ and we can number all the peaks measured according to the number of charges. *M* can be calculated from their mass. The average value obtained from all of the measured peaks is 17 827.9 Da with a mean error of 2.0 Da. This technique allowed the determination of the molecular masses of proteins above 130 kDa with a detection limit of about 1 pmol using a quadrupole analyser.

A variety of algorithms have been developed to allow the determination of the molecular mass through the transformation of the multiply charged peaks present in the ESI spectrum into singly charged peaks. Some of them also allow the deconvolution ESI spectra of mixtures, as is shown in Figure 1.24. However, the complexity of the spectra obtained for a single compound is such that only simple mixtures can be analysed.

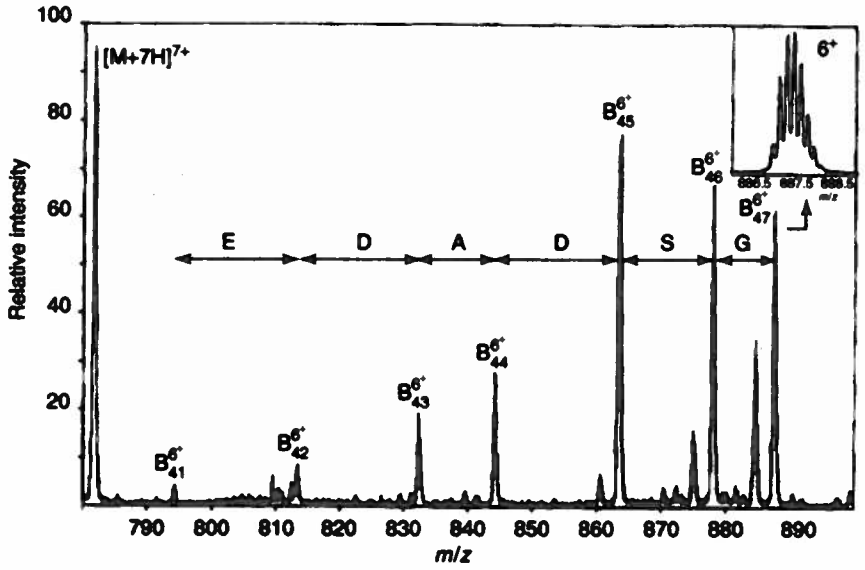

Figure 1.25
Product ion spectrum of the $[M + 7H]^{7+}$ ion from the following peptide: ALVRQGLAKVAYVYKPNNTHEQHLRKSEAQAKKEKLLNIWSEDNADSGQ. Notice that fragment ions having lower charge number z may appear at higher m/z values than the precursor, which indeed occurs in the spectrum shown. The inset shows that, owing to the high resolution, the isotopic peaks are observed separated by 1/6 Th, and thus $1/z = 1/6$ or $z = 6$. From Andersen J., Molina H., Moertz E., Krogh T.N., Chernuchevich I., Taylor L., Vorm O. and Mann M., 'Quadrupole-TOF Hybrid Mass Spectrometers Bring Improvements to Protein Indentification and MS/MS Analysis of Intact Proteins' The 46th Conference on Mass Spectrometry and Allied Topics, Orlando, Florida, 1998, p. 978. Reprinted, with permission.

At high resolution, the individual peaks with different charge states observed at low resolution are each split into several peaks corresponding to the isotope distribution. As neighbour peaks differ by 1 Da, the observed distance between them will be $1/z$, allowing the direct determination of the charge state of the corresponding ion. This is important for MS/MS spectra of multiply charged ions, as the preceding rules to assign the z value can no longer be applied. An example is displayed in Figure 1.25 [67].

The ability of this ionization method for the determination of very high molecular weights is illustrated in Figure 1.26 [68]. The spectrum displayed is obtained from assemblies of vanillyl alcohol oxidase containing respectively 16 and 24 proteins. The spectrum was obtained with a hybrid quadrupole TOF instrument, Q-TOF Micromass, equiped with a micro-ESI source. To obtain such a spectrum one needs not only a mass spectrometer with sufficient mass range and resolution, but also high skill in protein purification.

1.11.2 Electrochemistry and Electric Field as Origins of Multiply Charged Ions

Charges of ions generated by ESI do not reflect the charge state of compounds in the analysed solution, but are the result of both charge accumulation in the droplets and charge

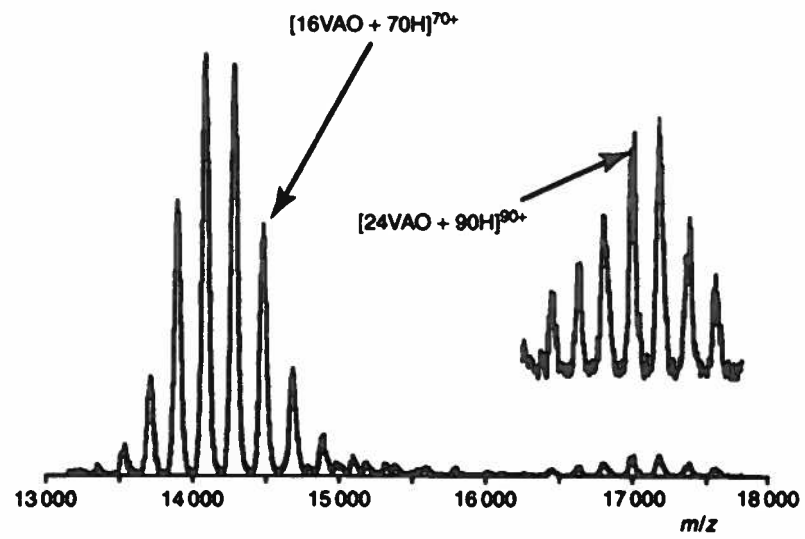

Figure 1.26
MicroESI Q-TOF spectrum of assemblies of vanillyl alcohol oxidase obtained by W.J.H. van Berkel and co-workers [68]. At the left, an octamer–dimer of 1.02 MDa molecular weight, and at the right, the octamer–trimer of 1.53 MDa. Reproduced courtesy of Dr. A.J.R. Heck.

modification by electrochemical process at the probe tip. This is clearly demonstrated by a convincing experiment reported by Fenselau and co-workers, and illustrated in Figure 1.27 [69]. They showed that negative ions of myoglobin can be observed at pH 3, while a calculation based on known pK values predicts that only 1 molecule per 3500 approximately would have one negative charge in the original solution. The results point out the role of the charge accumulation in droplets under the influence of the electric field on the formation of multiply charged ions. Furthermore, the 'pumping out' of the negative charges can only be performed if, at the same time, the same number of positive charges is electrochemically neutralized at the probe tip.

Moreover, it is worth noting that the negative ion spectrum of myoglobin at pH 3 shows a better signal-to-noise ratio than the same spectrum at pH 10 (Figure 1.27). This results from the fact that protons have a high electrochemical mobility, and is the first indication of the importance of the reduction process, when negative ions are analysed, that occurs at the probe tip.

At pH 10, positive ions can be observed too. Additional peaks in the spectra result from a modification of the protein at basic pH (loss of heme group).

Thus, it is worthy of consideration to try to acidify a solution with a view to a better detection of negative ions, and vice versa. Indeed, both H_3O^+ and OH^- have high limit equivalent conductivities, as shown in Table 1.3.

1.11.3 Sensitivity to Concentration

Another feature of ESI is its sensitivity to concentration, and not to the total quantity of sample injected in the source, as is the case for most other sources. This is shown in

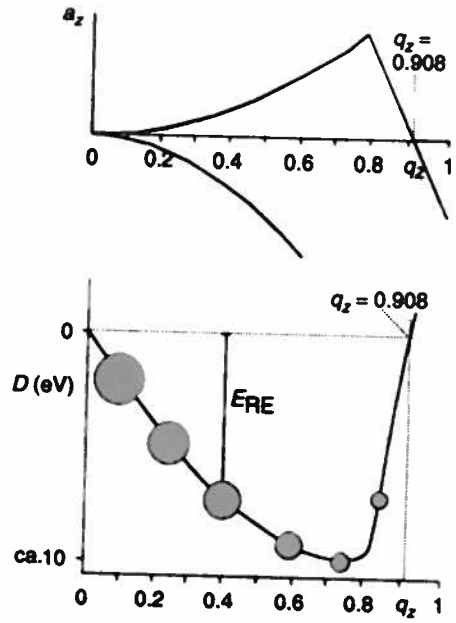

Figure 2.27
Dehmelt potential D in electronvolts (eV) (lower graph) as a function of q_z. Heavier ions, for a given V value, have lower Dehmelt trapping energies. An increase in V will displace all the ions along the curve to higher q_z values. If q_z passes the 0.908 value, the ion will be expelled. The E_{RE} line represents the energy that has to be provided by resonant excitation in order to expel the selected ion from the trap. The top graph resembles the stability diagram. The ordinate is not the same for the two diagrams.

residue with the *N*-acetylglucosamine (Gal(β1–4) GlcNac(β1–3) Gal(β1–3) Glc). The spectra show that the two different terminal disaccharides can easily be distinguished [18]. In this paper it is also shown that these same disaccharides originating from larger oligosaccharides yield the same fragmentation spectra, even if several fragmentation steps are used, for instance MS^4, MS^5 or more, provided the collision energy in the last step is the same.

2.2.1.8 Space Charge Effect

When too many ions are introduced into an ion trap, those located at the outside will act as a shield. The field acting on the ions located at the inside will be modified, and the shape of the stability diagram will be modified as displayed in Figure 2.30.

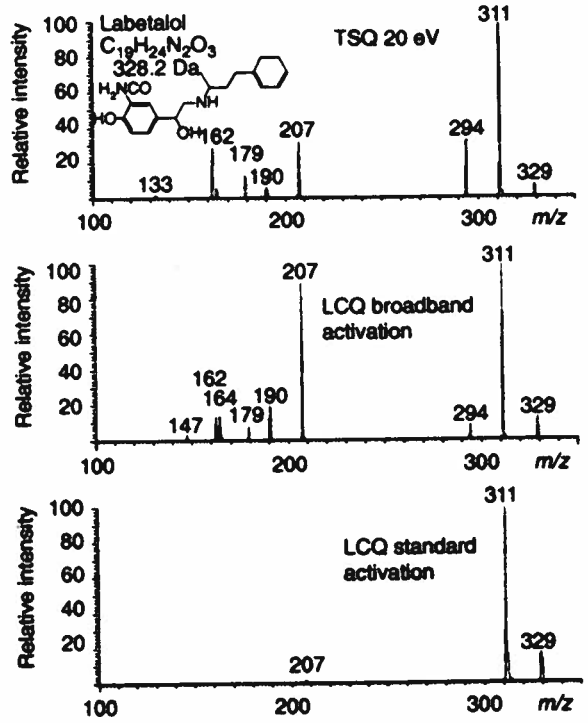

Figure 2.28
Top: fragment ion obtained from ESI protonated Labetalol molecular ion in a triple quadrupole instrument. Centre: same spectrum obtained in an ion trap with broadband excitation. All the ions, precursor or fragment, are activated by resonant irradiation. Bottom: same spectrum as obtained with an ion trap when only the precursor ion is activated. Redrawn from Senko M.W., Cunniff J.B. and Land A.P., 'Producing "Richer" Product Ion Spectra on a Quadrupole Ion Trap with Broadband Activation', Proceedings of the 46th ASMS Conference on Mass Spectrometry and Allied Topics, Orlando, Florida, p. 486, 1998, with permission.

2.2.2 The 2D Ion Trap

2.2.2.1 General Principle

The 2D ion trap, also known as the linear ion trap (LIT), is an analyser based on the four-rod quadrupole ending in lenses that repel the ions inside the rods, and thus at positive potentials for positive ions, and vice versa. In these traps, ions are confined in the radial dimension by means of a quadrupolar field and in the axial dimension by means of an electric field at the ends of the trap.

The LIT principles were recently reviewed by Douglas [19]. Once in the LIT, the ions are cooled by collision with an inert gas and fly along the *z* axis between the end electrodes,

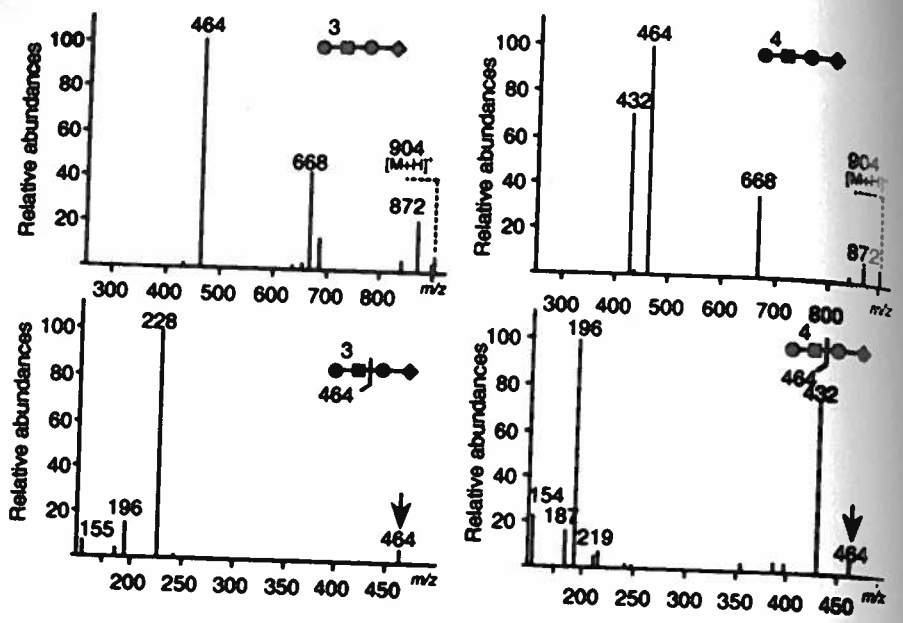

Figure 2.29
MS^2 and MS^3 mass spectra from two identical oligosaccharides except for the position of the first galactose residue on the *N*-acetylglucosamine. These same disaccharides originating from more complex oligosaccharides produce these same fragmentations. Reproduced (modified) from Viseux N., de Hoffmann E. and Domon B., Anal. Chem., 69, 3193–3198, 1997, with permission.

while simultaneously oscillating in the *xy* plane owing to the application of an RF-only potential on the rods. The rods are furthermore often divided into three segments. Application of an additional DC voltage to the end parts of the quadrupole also allows the ions to be trapped. This can be used either without end electrodes or together with these electrodes.

These voltages repel the ions inside the linear trap, and this repulsion is higher when the ions are closer to the ends. Ions are thus repelled towards the centre of the quadrupole if the same repelling voltages are applied at each end. Thus, the ion cloud will be squeezed at the centre of the quadrupole if the applied voltages are symmetrically applied, but can be located at closer to one end if the repelling voltage at that end is smaller.

One great advantage of LITs in comparison with Paul ion traps is a more than 10-fold higher ion trapping capacity. Furthermore, this higher trapping capacity is combined with the ability to contain many more ions before space charge effects occur owing to both a greater volume of the trap and a focusing along the central line rather than around a point. Even with 20 000 trapped ions, well resolved mass spectra can be obtained without any space charge effects in 2D ion traps, whereas more than 500 trapped ions in 3D traps induce space charge effects. Another common advantage is the higher trapping efficiency. Indeed, a trapping efficiency of more than 50 % is achieved when ions are injected into the 2D ion trap from an external source, while this trapping efficiency is only 5 % for the 3D ion trap. Both of these advantages increase the sensitivity and the dynamic range.

Ions trapped within an LIT can be mass selectively ejected either along the axis of the trap (axial ejection) or perpendicular to its axis (radial ejection). Therefore, in commercial

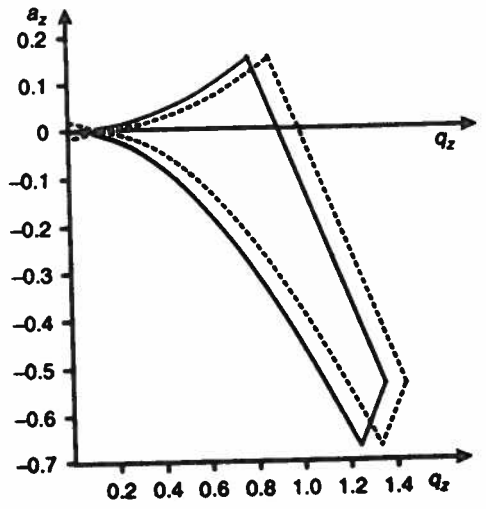

Figure 2.30
The dashed line represents the shift of the stability diagram resulting from the space charge effect. To reach the stability limit $\beta = 1$, q_z, and thus V, has to have a higher value. This could lead to an error on the mass if proper caution is not taken.

LITs two modes for the mass selective ejection of ions are used: either the ions are expelled axially using fringe field effects by applying AC voltages between the rods of the linear trap and the exit lens, or slots are hollowed out in two opposite rods and mass selective radial expulsion of ions is obtained by applying an appropriate AC voltage on these two rods.

2.2.2.2 *Axial Ejection in Linear Trap*

The linear trap with axial ejection was invented by Hager, from MDS Sciex, in 2002 [20]. Figure 2.31 displays a scheme of such an ion trap included in the ion path of a triple quadrupole mass spectrometer.

As shown in Figure 2.32, the ions are expelled axially using fringe field effects by applying AC voltages between the rods of the linear trap and the exit lens.

This instrument can be operated as a normal triple quadrupole with all its scan modes or as a trap in various combinations with the use of the other quadrupoles. If a slow scan rate is used to expel the ions a resolution up to 6000 FWHM can be reached by scanning at 5 Th s^{-1} using q_2 and at 100 Th s^{-1} using Q_3, which is at a lower pressure. As fringe field effects are used, only ions close to the exit lens are expelled. In consequence, mass selective ejection in the axial direction based on this technique is characterized by low ejection efficiency. For instance, an ejection efficiency of less than 20 % is achieved at 1 Th ms^{-1} scan rate. Different techniques have been proposed to improve the axial ejection efficiency [21], but the most promising technique for mass selective axial ejection is the technique named axial resonant excitation (AREX) [22]. Lenses are introduced between each rod of the quadrupole

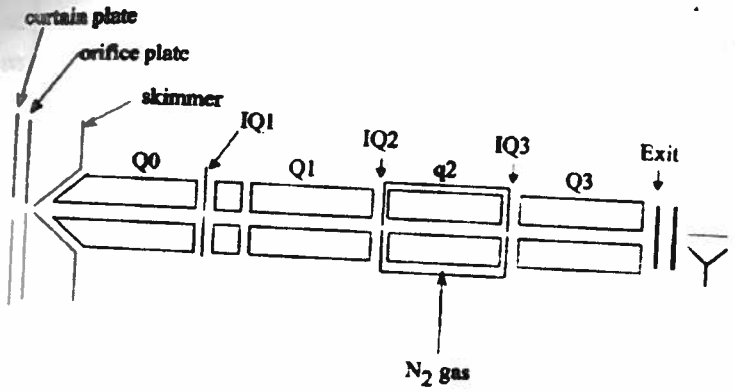

Figure 2.31
View of the linear trap included in a triple quadrupole at q2. This instrument can be operated as a regular triple quadrupole or with a trap. Reproduced from Hager J.W., Rapid Comm. Mass Spectrom., 16, 512–526, 2002, with permission.

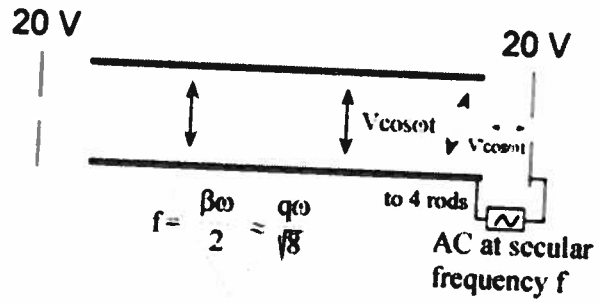

Figure 2.32
Imaged view suggesting the principle that, near the exit, the trajectory of the ions has a component along the axis at the same frequency as the radial oscillation. This can be used to expel the ions by applying an AC voltage at the same frequency between the quadrupole and the exit lens or by increasing the RF amplitude V (radial excitation).

to induce an electrostatic potential that is approximately harmonic along the central axis of the quadrupole. Inside this potential, ions with a given m/z can oscillate resonantly in the axial direction by superposing a supplemental AC field. High ejection efficiency has been observed. For instance, an ejection efficiency of more than 60 % is achieved at 10 Th ms^{-1} scan rate, three times more than with the technique using a fringe field. However, in these conditions, the mass resolution is about 1000.

2.2.2.3 *Radial Ejection in Linear Trap*

Radial ejection between the rods has been described [19] but never applied to commercial instruments. Ejection through slots cut in two opposite rods was described first by Senko and Schwartz from Thermo Finnigan in 2002 [23, 24].

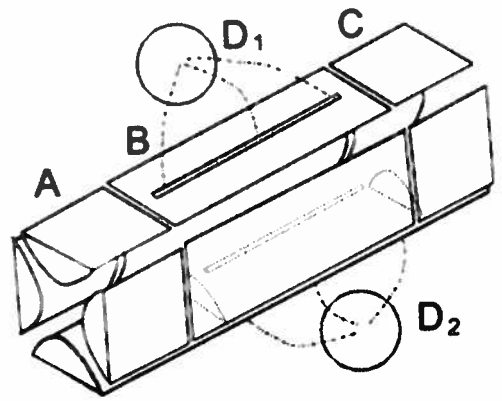

Figure 2.33
Linear trap with slots cut in two opposite rods. Sizes are 12 mm for sections A and C and 37 mm for B. Detectors D are placed off-line and ions are attracted by the conversion dynodes. The slots are 30 × 0.25 mm. Drawn according to the data from Schwartz J.C., Senko M.W. and Syka J.E.P., 'A Two-Dimensional Quadrupole Ion Trap Mass Spectrometer', Proceedings of the 50th ASMS Conference on Mass Spectrometry and Allied Topics, Orlando, Florida, 2002.

Figure 2.33 represents such a linear trap. The two detectors allow the use of all the ions expelled from the trap. Trapping efficiency is in the range 55–70 % while it is only 5 % in the Paul ion trap. Unit resolution is achieved at 16 700 Th s^{-1} scan rate. At 27 Th s^{-1}, $\Delta m = 0.05$ is observed at m/z 1520, corresponding to a resolution of 30 000 FWHM. The ion capacity is about 20 000, 40 times more than in the Paul ion trap.

The presence of the slots causes a perturbation of the RF field that can be reduced by slightly stretching the quadrupole, increasing the distance between the cut rods.

Mass selective ejection of the ions in a radial direction occurs by applying an AC voltage between the two cut rods. As for the 3D ion trap, an AC frequency corresponding to $q_z = 0.88$ is used. Ions of successively higher masses are brought to this q_z value by increasing V. An ejection efficiency of about 50 % is achieved at 5 Th s^{-1} scan rate [25].

Operations similar to the 3D traps can be performed, as for example to expel ions of all masses except one and observe the fragmentation, with or without ion excitation at the secular frequency. Then the fragments are analysed. This can be repeated several times for MS^n experiments. All the other operations of a 3D trap can be applied, but it also has similar limitations, for example MS/MS is limited to fragmentation scans. Thus precursor ion scan or neutral loss scan that are available with triple quadrupole instruments cannot be used on ion traps (Figure 2.11).

Segmented quadrupoles are used rather than trapping by end electrodes, to avoid producing fringe fields. To trap the ions, a DC voltage is applied to the end sections. Coupling to the source occurs through an interface comprising focusing multipoles, as described for the 3D ion trap.