

10 Time of Flight Mass Spectrometry (TOF-MS)

Mass spectrometry is a technique used to separate, and to measure the (relative) masses of, ions produced in the gas phase under high-vacuum conditions. Both the time-of-flight (TOF) mass spectrometer and the conventional magnetic-deflection mass spectrometer involve the same four processes: ionisation, acceleration, analysis and detection; and both require very high vacuum conditions for their accurate operation. Their main difference lies in the way that the beam of ions is separated and analysed.

In the TOF mass spectrometer separation is achieved by allowing the ions to pass through an electric field-free zone between their acceleration and their detection. If the ions have all been given the same kinetic energy by the accelerating voltage, their velocities, and hence the time it takes them to traverse the field-free zone, will differ according to their masses. The heavier ions will move more slowly, so will take longer to reach the detector.

$$t = \frac{d}{\sqrt{2eE}} \sqrt{m/z} \quad [1]$$

where t = time; d = length of field-free zone; e = fundamental electronic charge; E = accelerating potential; m = mass of ion; z = charge on ion.

[The appendix to this article shows a derivation of this equation.]

[Q: candidates could be asked to derive this themselves]

The features of the instrument

The physical design of the TOF mass spectrometer, and in principle its electronic circuitry, are both more simple than that of a conventional mass spectrometer.

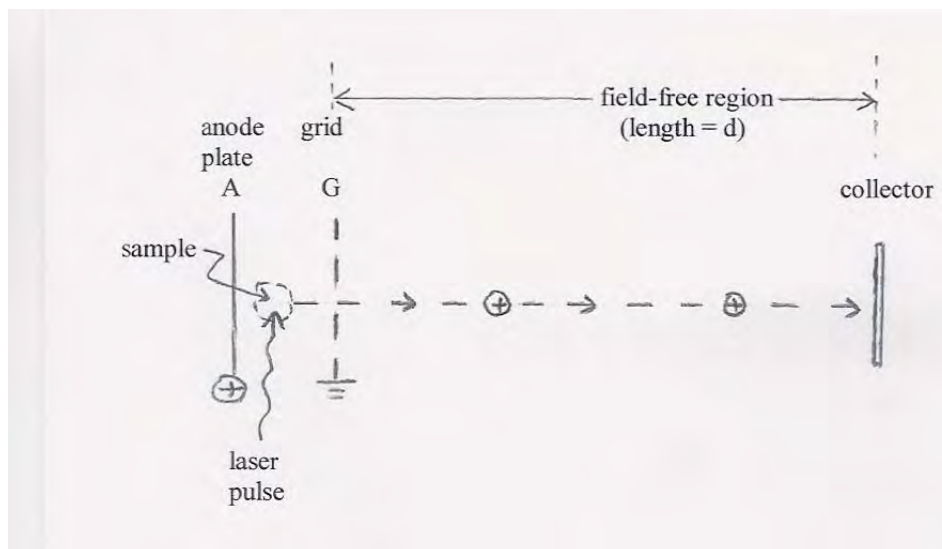


figure 1 a linear TOF mass spectrometer

Accurate TOF mass spectrometry is highly reliant on the accurate measurement of time, so progress in TOF spectrometry had to wait for the development of sensitive time-to-digital converters. New techniques of ionisation have also been developed, since in

several types of spectrometer the instantaneous time of ionisation needs to be known with relation to the time of detection. Laser pulses are often used to effect ionisation.

Some rough dimensions and values

Using equation [1], we can calculate the time of flight for a typical molecule-ion.

In many TOF mass spectrometers, the path length, d , of the field-free zone is about 1.5 m and the accelerating voltage is often about 5000V. Let us assume the molar mass of the sample to be about 500 a.m.u. Taking $e = 1.6 \times 10^{-19}$ C and $m_H = 1.67 \times 10^{-27}$ kg (mass of hydrogen atom), and assuming a singly-charged molecular ion ($z = 1$), we can calculate t as follows:

$$t = [1.5/\sqrt{(2 \times 1.6022 \times 10^{-19} \times 5000)}] \times \sqrt{(500 \times 1.6736 \times 10^{-27})} = 3.428 \times 10^{-5} \text{ s}$$

The corresponding time for an ion of mass 499 (i.e. 1 a.m.u. lower) is 3.425×10^{-5} s, so if we need the spectrometer to distinguish between these two ions, the electronic circuitry must be capable of measuring to an accuracy of 0.003×10^{-5} , i.e. 3×10^{-8} s.

[Q: candidates could be asked to calculate the time for $m/e = 499$ and hence work out the sensitivity required]

The advantages of the TOF mass spectrometer over a conventional instrument

- its cost is often less than that of a conventional instrument
- only small amounts of sample are needed to obtain the mass spectrum: fractions of a femtogram (1×10^{-15} g) or even attogram (1×10^{-18} g) quantities can be used.
- theoretically, the mass range that can be analysed is virtually unlimited, so the technique has found great use in analysing large biological macromolecules with high M_r .
- it is much lighter than a conventional instrument, and can be constructed to be much smaller too, so it is ideally suited for use in spacecraft and interplanetary probes.

The spectrometer described in greater detail

A. How the ions are formed

Both because the time of ionisation often needs to be known accurately, but also because the substances to be analysed can be fairly unstable biological molecules, novel methods of ionisation have been developed. Two of them will be described here.

a. *Electrospray ionisation*

In this technique a solution of the sample is ejected through a small-diameter tip of a capillary tube. The tip is kept at a high positive potential of a few thousand volts. While the solution passes through the tip, electrons are removed from it and a spray of positively-charged droplets emerges. As the solvent evaporates, the droplets become

smaller and smaller, and their surface charge density increases until the electrostatic repulsion between touching ionic charges causes a “coulombic explosion” to occur, forming even smaller droplets. Eventually, all the solvent molecules will have evaporated, leaving ionised molecules of the sample in the gas phase.

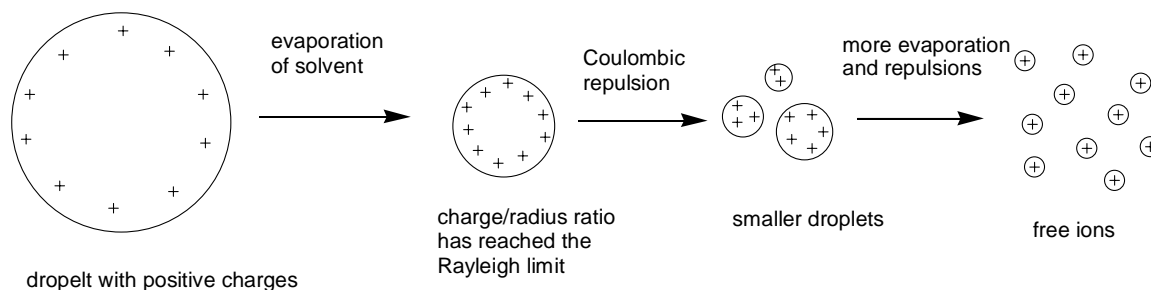


figure 2 the break-up of an electro spray

b. *Matrix-assisted laser desorption ionisation (MALDI)*

In this technique the sample and the matrix compound are co-dissolved in a solvent (the ratio is often about 1:1000 by mass). Spreading the solution onto a solid stainless steel plate and allowing the solvent to evaporate produces a solid crystalline matrix containing a small proportion of the sample. When the surface of the matrix is exposed to a short pulse of laser light (tuned to the wavelength of maximum absorbance of the matrix molecule), positively-charged ions of the sample are produced (along with many neutral molecules, which are readily removed via the vacuum pump). The ions are often radical-cations (formed by the loss of an electron from a lone pair on the sample molecule) or protonated molecular ions (formed by H^+ being transferred from an ionised matrix molecule to the sample molecule). The compounds used as the matrix need to have various properties. They need to absorb strongly at the wavelength range of the laser (lasers cannot be tuned over too large a range); they need to be inert with respect to the sample; they need to be fairly involatile; but they need to be fairly soluble in the same solvents that dissolve the substrate (often water). Three common ones are nicotinic acid, 2,5-dihydroxybenzoic acid and 3,5-dimethoxy-4-hydroxycinnamic acid.

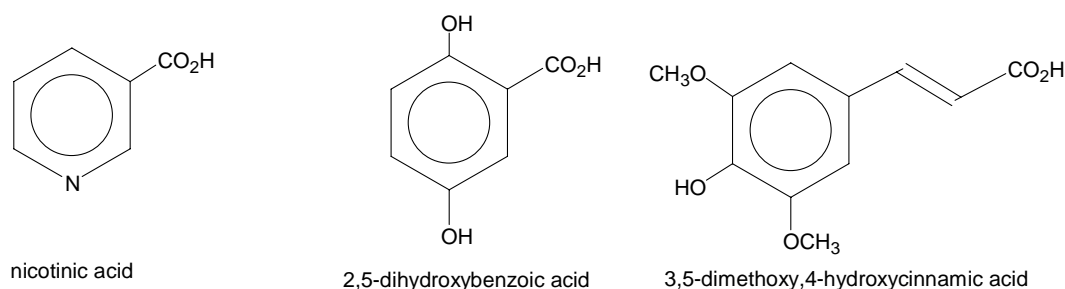
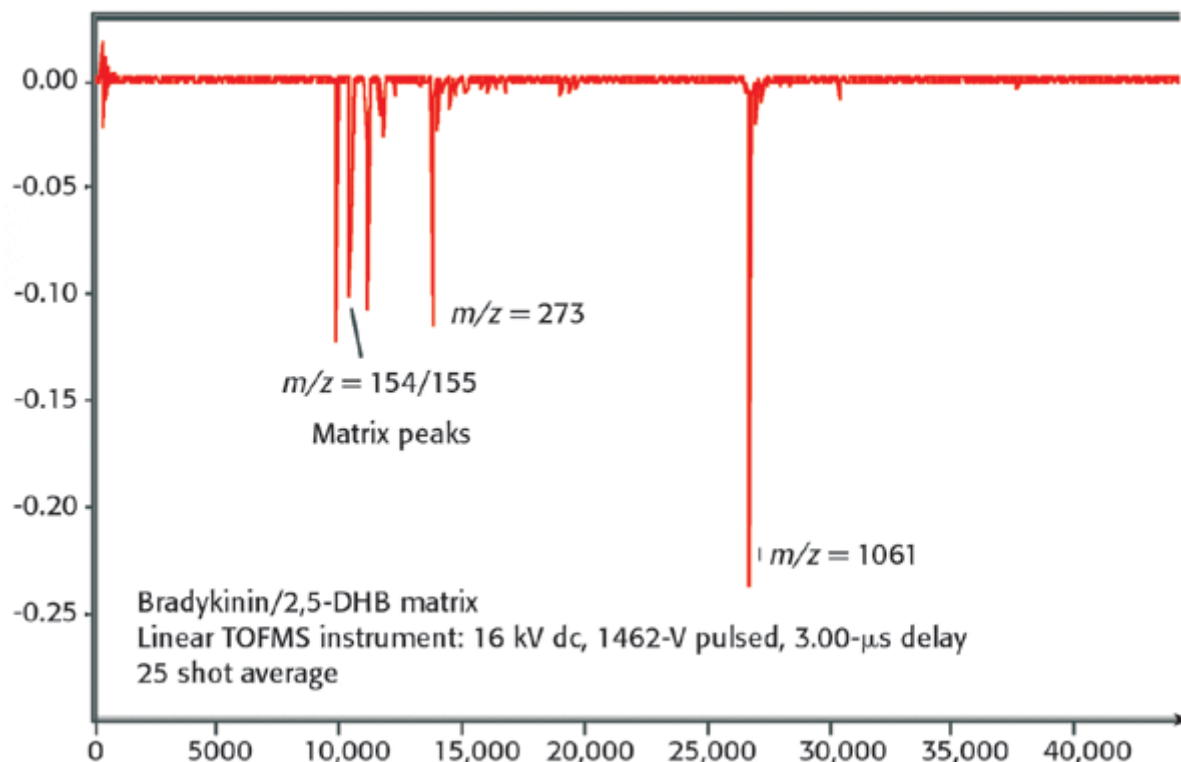


figure 3 some compounds used as matrices

Ions derived from the matrix compound will also appear in the mass spectrum, but these are easily identified. Also, ions derived from the sample are usually of much higher M_r than those derived from the matrix compound, so little interference results (see figure 4)



(from <http://spectroscopymag.findpharma.com/spectroscopy/Mass+Spectroscopy/Time-of-Flight-Mass-Spectrometry-for-Proteomics-Re/ArticleStandard/Article/detail/161412>)

Figure 4 MALDI mass spectrum of bradykinin.

B. The acceleration process

Once formed, the ions are accelerated by applying a positive potential (5 – 15 kV) to the anode plate A (figure 1), relative to grid G. This potential repels the positive ions and they pass through the grid G into the field-free region.

As mentioned before, it is important that the exact time at which the ions enter the field-free region is known. There are two ways of achieving this.

- The accelerating voltage is applied continuously, and the “clock” is started at the exact time that the pulsed laser responsible for ionisation is turned on.
- In the “delayed extraction” method, the sample is ionised and the resulting small plasma cloud is then allowed to equilibrate and expand for a small time (a fraction of a microsecond). A pulse of accelerating voltage is then applied, at the same time as the “clock” is started.

The second method allows a better focussing of the ion beam, and is the one usually used with the MALDI method of ionisation.

C. The analyser

In the simple “linear” TOF spectrometer, the analyser is merely a length of tube, evacuated to a very low pressure, through which the ions travel towards the detector at the right hand end (see figure 1). However, the line-resolution of the linear TOF spectrometer is not very high. Ions of the same mass can arrive at the detector at slightly different times, for three possible reasons.

- a. The ions may not all have been formed at exactly the same time – some may have been formed a few nanoseconds after others. Errors due to this temporal spread can be eliminated by the “delayed extraction” method of acceleration, wherein ALL ions are accelerated at the same time, no matter when they were formed.
- b. The ions may not all start at the same place – some may be on the left of the source, near the accelerating plate A, whilst others may start near the grid G. Once the accelerating potential is applied, although those to the left will have a greater length to travel to the detector, they will however experience the accelerating potential for a greater time, and so will pass through the grid G at a faster speed than those ions that started near to grid G.
- c. The ions may not all have exactly the same kinetic energy as each other. For ions of the same mass number, this means that some will be travelling at a faster speed than others. Once formed, the positive ions in the plasma will repel each other. Some ions will be moving toward the left, whilst others will be moving towards the right. The former will not pick up as much speed as the latter when the accelerating potential is applied.

The reflectron

Ions with the same mass number travelling at different speeds can still arrive at the detector at the same time if a reflectron is used. This is a series of positively charged plates that repel the ion beam, and “reflect” it back towards the detector, which is placed slightly to one side of the un-reflected ion beam. (see figure 5). Ions that are travelling faster will penetrate the field within the reflectron more, before they are turned back. Their path-length from grid G to the detector will therefore be longer than the path-length of ions that are moving more slowly. The instrument geometry and the reflectron potentials can be adjusted so that all ions of the same mass number will eventually reach the detector at the same time.

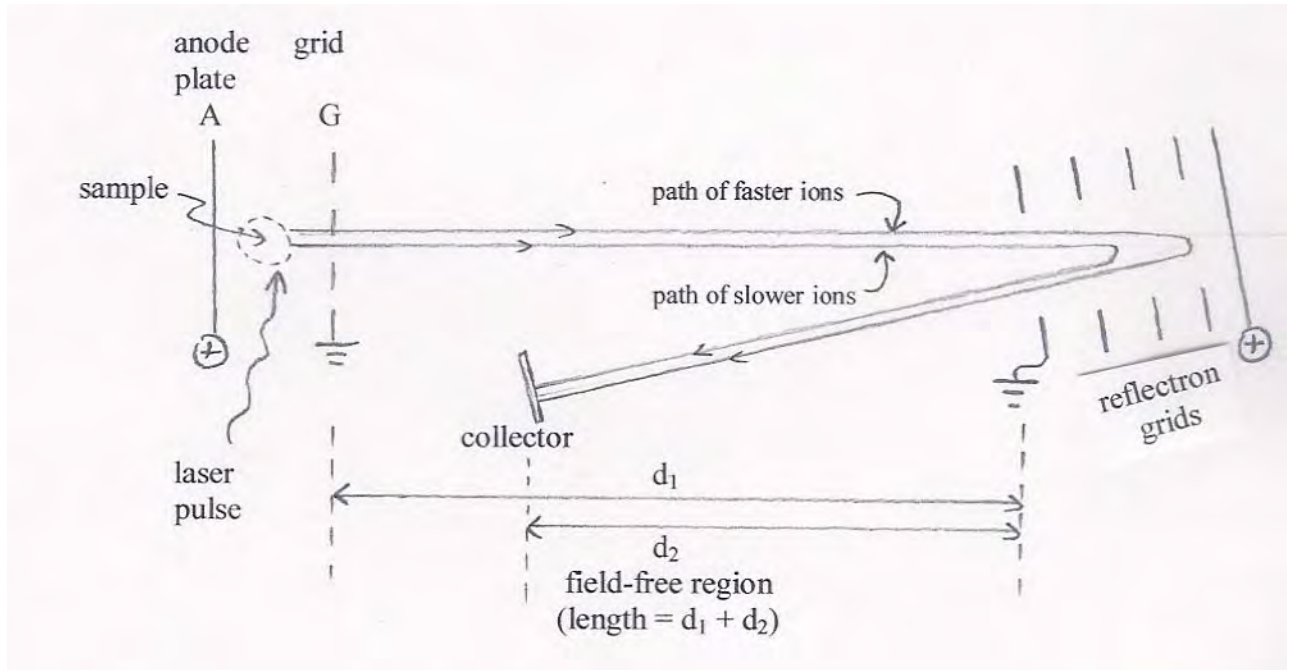


figure 5 a reflectron-TOF mass spectrometer

Figure 6 shows the greatly increased resolution obtained by using a reflectron: the 1 in 600 resolution of the linear instrument has been increased to 1 in 3400.

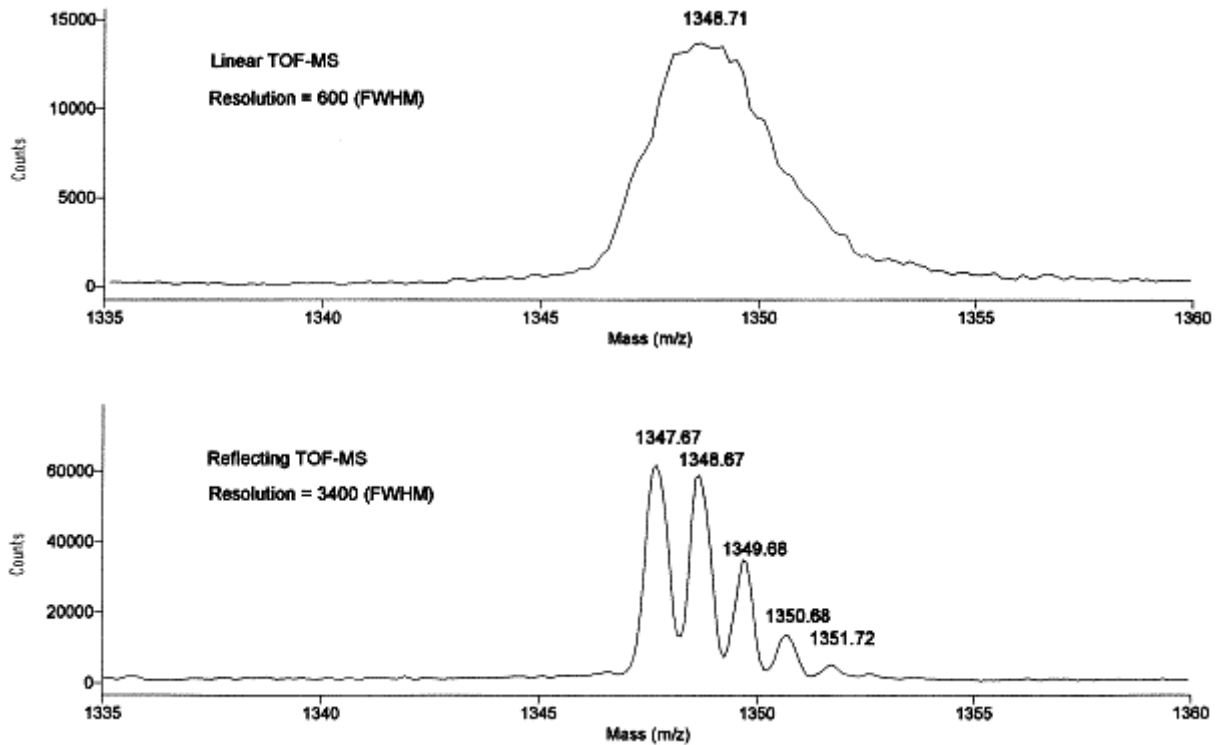


Figure 6 Demonstration of the increased resolution achieved by the reflectron (from <http://www.abrf.org/ABRFNews/1997/June1997/jun97lennon.html>)

D. The detector

This is usually a microchannel plate coupled to a time-to-digital converter (essential a counting device that measures the time elapsed since the acceleration of the ion beam). The specification of one such detector is as follows (<http://www.rmjordan.com/mcpdetector.html>). “Designed to handle the fast ion pulses provided by our linear and reflectron Time Of Flight Mass Spectrometers, the MCP Microchannel Plate Detector has a 50 ohm coaxial output and provides high gain (1×10^6 to 1×10^7) with sub-nanosecond rise times.”

Applications of TOF Mass Spectrometry*a. The analysis of biological macromolecules.*

Making use of its ability to deal with ions of high m/z values, TOF-MS has not only been used to identify chemically large M_r compounds in biological samples, but also to deduce the structures of small protein fragments by the technique of “ladder” sequencing. This is illustrated in figure 7, which shows the spectra obtained from a series of partial hydrolyses of the adrenocorticotrophic hormone (ACTH) fragment 18-39 by carboxypeptidase-Y (CPY) at various concentrations. The bottom spectrum is that of the ACTH fragment with no CPY added, and the other spectra are of hydrolysates derived from mixtures containing increasing [CPY]. The amino acid sequence can be read off by noting the difference in m/z values of adjacent peaks.

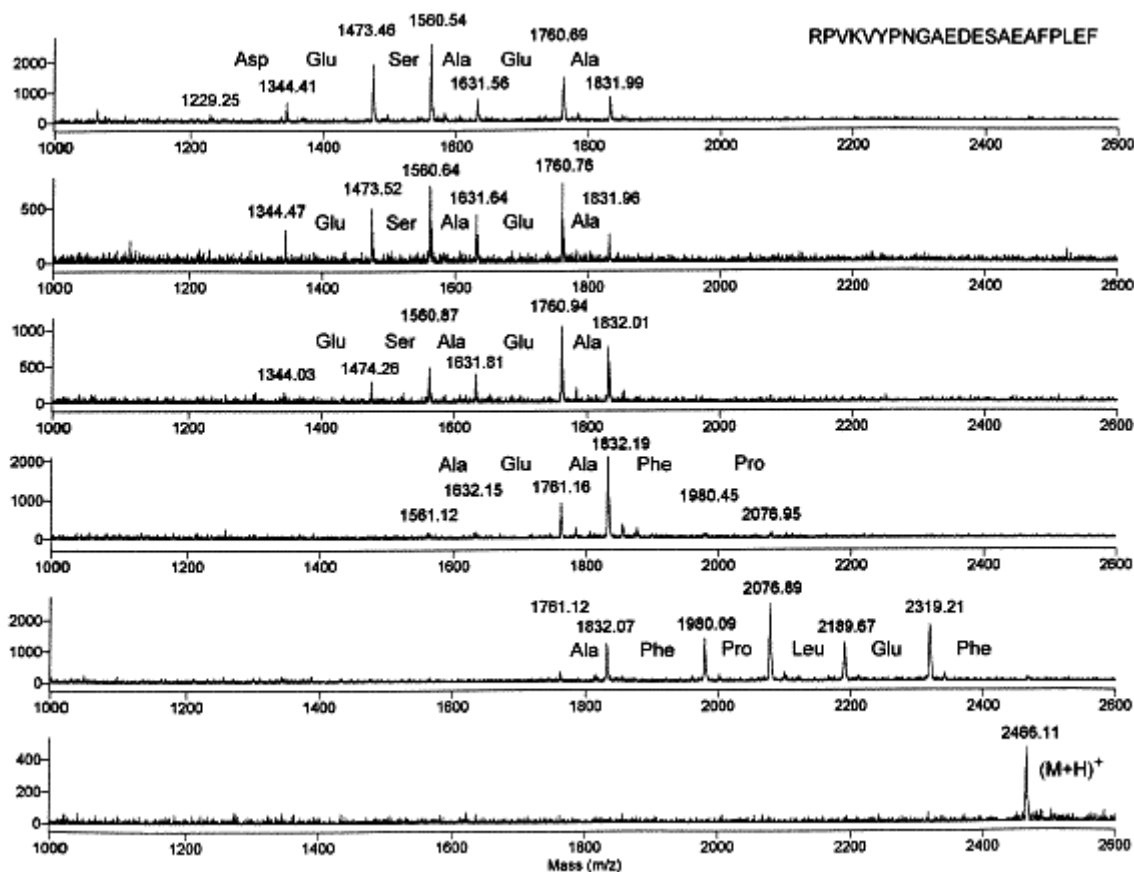


Figure 7 Ladder sequencing of ACTH fragment 18-39
(from <http://www.abrf.org/ABRFNews/1997/June1997/jun97lennon.html>)

Interpretation of the spectra in figure 7 allows the sequence of the first 11 amino acids from the $-CO_2H$ end of the peptide to be determined. Further exposure to CPY would cause more hydrolysis, and allow the sequence of the other 11 amino acids of the fragment to be determined. [Q: candidates could be asked to work out the sequence of the first 11 amino acids]

b. Space exploration.

Although unmanned probes sent to sample the atmospheres of other planets and moons in the solar system have usually employed quadrupole mass spectrometers, small lightweight TOF spectrometers are being developed to enable in-craft analysis of astronauts' biomarkers, to monitor their health on long space voyages. The following passage describes work carried out for NASA by Johns Hopkins University (<http://www.nsbri.org/Research/Projects/viewsummary.epi?pid=108>).

"At present, identification of each biomarker requires a separate protocol. Many of these procedures are complicated and the identification of each biomarker requires a separate protocol and associated laboratory equipment. To carry all of this equipment and chemicals on a spacecraft would require a complex clinical laboratory, and it would occupy much of the astronaut's time. What is needed is a small, efficient, broadband

medical diagnostic instrument to rapidly identify important biomarkers for human space exploration.

The Miniature Time-Of-Flight Mass Spectrometer Project in the Technology Development Team is developing a small, high resolution, time-of-flight mass spectrometer (TOFMS) to quantitatively measure biomarkers for human space exploration. Virtues of the JHU/APL TOFMS technologies reside in the promise for a small (less than one cubic ft), lightweight (less than 5 kg), low-power (less than 50 watts), rugged device that can be used continuously with advanced signal processing diagnostics. To date, we have demonstrated mass capability resolution from under 100 to beyond 10,000 atomic mass units (amu) in a very small, low-power prototype for biological analysis. Further, the electronic nature of the TOFMS output makes it ideal for rapid telemetry to Earth for in-depth analysis by ground support teams.”

[further Q: candidates could be asked to carry out internet searches to describe more applications of TOF-MS, different ionisation techniques, and also to present short papers on how the detector works, or the physics of the reflectron]

Appendix: The theory of mass spectrometry

In both the conventional magnetic-deflection mass spectrometer and the TOF mass spectrometer, the ions are separated according to their mass-to-charge ratio, m/z . Once formed, the ions, having an electrical charge of q coulombs, are accelerated by an electrical potential of E volts. Being in the gas phase and free of any constraints, the potential energy they have gained, qE , is converted into kinetic energy, $\frac{1}{2}mv^2$ (m = mass of ion; v = velocity).

$$qE = \frac{1}{2} mv^2 \quad [1]$$

A. The physics of the conventional magnetic-deflection spectrometer.

(This is included mainly for completeness, and by way of a comparison with the equations for the TOF instrument)

The conventional mass spectrometer uses the fact that a beam of ions moving with a velocity v will experience a sideways force as it traverses a magnetic field, given by the equation

$$\text{force} = Bqv. \quad [2]$$

As this force acts at right angles to its direction of motion, the ion will follow a circular path, the normal acceleration being v^2/r , and the force being given by the equation

$$\text{force} = mv^2/r. \quad [3]$$

Combining the three equations [1] – [3] gives the required relationship between m/z , the field B and the radius of the circular path, r .

$$Bqv = mv^2/r \quad \Rightarrow \quad B^2q^2v^2 = m^2v^4/r^2 \quad \Rightarrow \quad B^2r^2v^2 = (m^2v^2/q^2)(v^2)$$

$$\Rightarrow \quad B^2 r^2 = 2(m/q^2)(\frac{1}{2}mv^2) \quad \Rightarrow \quad B^2 r^2 = 2(m/q^2) qE \quad \Rightarrow \quad (m/q) = \frac{B^2 r^2}{2E}$$

Replacing the charge q by ze , where z is the charge on the ion in units of e , the fundamental electron charge, we have the relationship

$$m/z = B^2 r^2 e / 2E$$

Thus, if E and B are constant, ions with different m/z ratios will follow paths with different radii r , or (as is more usually the case in a conventional mass spectrometer) ions with different m/z ratios will require different a magnetic field strength B to keep constant the radius of their path through to the detector.

B: The physics of the Time-of-Flight Mass Spectrometer

There is no magnetic field in the TOF spectrometer. Instead, once the ions have been accelerated, the time they take to traverse a field-free region from the accelerator grid to the detector is measured. This time will depend on their velocity as follows:

$$v = d/t \quad \text{or} \quad t = d/v \quad [4]$$

where d is the length of the field-free region, and v is the velocity of the ions.

Combining this with equation [1], we have

$$qE = \frac{1}{2} mv^2 \quad \Rightarrow \quad qE = \frac{1}{2} m d^2 / t^2 \quad \Rightarrow \quad m/q = 2E t^2 / d^2$$

Replacing the charge q by ze , where z is the charge on the ion in units of e , the fundamental electron charge, we have the relationship

$$m/z = 2eEt^2/d^2$$

Thus, if E and d are constant, ions with larger m/z ratios will take longer to reach the detector. Rearranging this equation to make t the subject produces equation [1] on page 1.