The Medical and Biological Application of Mass Spectrometry

J. P. PAYNE, J. A. BUSHMAN
and D. W. HILL

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Edited by

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Preface

Almost ten years have passed since Fowler first specified the requirements of a mass spectrometer suitable for clinical use. Since then, a number of instruments have been developed and these have been used in a wide range of clinical conditions. In the light of this experience, it is now possible to review those features which are essential to a machine which will operate satisfactorily in a clinical environment and to indicate the applications which are most likely to be beneficial to patients and to advance research.

The knowledge which has been gained from past experience in the application of mass spectrometry in medicine can now be put to good use. Technically, both magnetic segment and quadrupole instrument are now easier to produce. Much more is known about both ion sources and detectors. Vacuum systems have become more reliable and compact, and the manufacturers have succeeded in producing safety interlocks which prevent catastrophic failures of the system despite the combined efforts of both clinical and technical staff.

The special requirements associated with the analysis of respiratory gases have been successfully met. The properties of the inlet system, together with its associated leak, are now well understood and it is possible to design systems with fast response times, including that for water vapour. Multiple inlet systems have also been designed and these are capable of measuring sequentially the gas exchange of a number of patients connected to a single mass spectrometer. Methods for measuring the blood gases *in vitro* and *in vivo* both intra-arterially and transcutaneously are now well advanced and some are even now undergoing clinical assessment.

The electronics have become very much smaller and more reliable with the advent of the integrated circuit. Recent work indicates that the clinical potential of the mass spectrometer can be greatly enhanced when run on-line to a computer and though for the present this must be regarded as a research project the advent of the microprocessor will allow the application of this work in a clinical environment within the next few years.

Apart from its direct application to clinical problems, mass spectrometry

has become virtually indispensible in a wide variety of disciplines including biochemistry, pharmacology and toxicology. In particular, it has become an analytical method of primary importance in the identification and structural analysis of organic compounds. For some problems, such as the identification of components separated by gas chromotography, it is one of the few techniques able to give specific data from a sample at the nanogram level.

This coupling of the gas chromatograph with the mass spectrometer has extended the applications of both techniques. The two methods are highly complementary to each other: the gas chromatograph can give excellent separation of the constituents of a mixture but not always a full identification, whereas the mass spectrometer can identify a single compound but is less efficient at identifying individual components in a complex mixture.

The accuracy and precision of this combined technique is further increased by the use of stable isotopes; as natural constituents of organic molecules, deuterium, carbon-13, nitrogen-15, and oxygen-17 and -18 offer important advantages in those clinical situations where radioactivity is best avoided. They are particularly suitable for the location and structural identification of metabolites and for the elucidation of biosynthetic pathways, and they are being increasingly widely used in studies of drug metabolism.

The papers presented in this monograph review these aspects, and others, and hopefully provide a definitive account of the present status of mass spectrometry in biology and medicine.

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Contents

1 The Evolution of Mass Spectrometers

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Although the earliest practical mass spectrometer was described by Dempster as long ago as 1918, it is interesting that many instruments in common use today follow closely his design. The principle of Dempster's magnetic deflection instrument is outlined in Fig. 1. This, like all mass spectrometers, operates in a vacuum system so that the influence of molecular collisions is negligible. The action starts in the ionization chamber, where positive ions are produced from gaseous molecules. These ions are all accelerated through the same potential difference, thus all gaining the same energy before being projected into a magnetic field as a slightly convergent beam. They move in a homogeneous magnetic field (perpendicular to the plane of the paper in Fig. 1) following circular

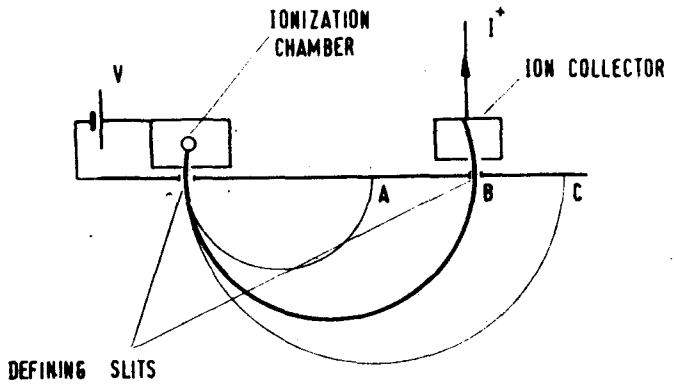

FIG. 1. The Dempster type mass spectrometer.

trajectories. The light ions are constrained to paths having small radii, the heavier ions following longer trajectories; the lightest ions being refocused at A and the heavy ions at C in Fig. 1. The ions refocusing at B pass through a defining aperture and are collected in a metal box (Faraday cage). The passage of the ions to the box creates an ion current, I^+, proportional to the ion beam intensity. If the field strength is increased the radius of each trajectory decreases. The pattern of trajectories therefore contracts and the heavy ions successively pass through the aperture and are collected. From this action we have the concept of a mass spectrometer.

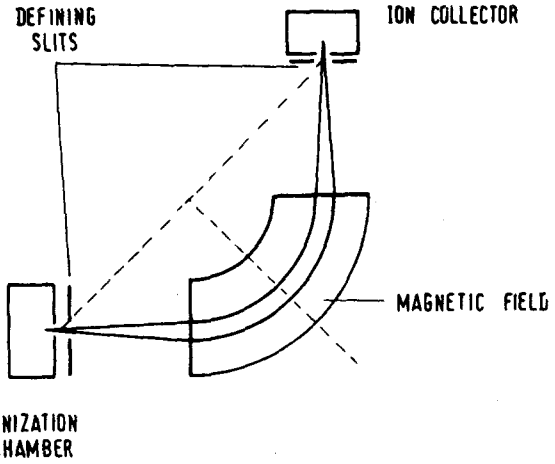

FIG. 2. The magnetic sector mass spectrometer.

The instrument outlined in Fig. 1 is expensive in magnetic materials so that further developments have led naturally to the sector field instrument, a typical example of which is shown in Fig. 2. The working principle is the same as the Dempster instrument. Molecules of the sample gas are ionized by electron bombardment within the ionization chamber, accelerated through a potential difference and projected in a slightly divergent beam into the electric field. In this case the field is defined precisely by a particular shaped pole piece. In the design illustrated, ions are deflected through 90° to refocus at the ion collector. Ions of a heavier mass will focus at points to the right of the collector defining slit, lighter ions focusing on the left.

With an instrument of the type illustrated in Fig. 2, ion beams can be separated with a resolution* of the order of 10 000. The limit to the

* A resolution of 10 000 means that ions differing in mass by one part in 10 000 can be distinguished.

resolution is set because instruments are truly momentum rather than mass analysers. In the discussion above, it has been assumed that all ions emerge from the source with precisely the same energy. This ideal condition is not achieved in practice so that ions having the same mass can have slightly different momentum, causing the focusing to be smeared rather like chromatic aberration in a lens system. The resolution cannot therefore be perfect, being limited in practice by the spread of momentum of ions of the

TABLE I. Eighteen compounds all with nominal mass 100 amu (Data from Kratos Ltd.)

Ion	Mass
$C_2H_2N_3O_2$	100.0466
$C_2H_4N_4O$	100.0705
$C_3H_2NO_3$	100.0354
$C_3H_4N_2O_2$	100.0592
$C_3H_6N_3O$	100.0831
$C_3H_8N_4$	100.1069
$C_4H_4O_3$	100.0480
$C_4H_6NO_2$	100.0718
$C_4H_8N_2O$	100.0956
$C_4H_{10}N_3$	100.1195
$C_5H_8O_2$	100.0844
$C_5H_{10}NO$	100.1082
$C_5H_{12}N_2$	100.1321
$C_6H_{12}O$	100.1208
$C_6H_{14}N$	100.1446
C_7H_2N	100.0507
C_7H_{16}	100.1572
C_8H_4	100.0633

same mass. Since the observation of ions of molecular mass greater than 1000 is rarely required, it would seem that the instrument outlined in Fig. 2 would meet all needs. In practice, however, there are many different compounds that have almost identical molecular masses. Table I lists 18 different molecules having the same nominal mass of 100 amu (atomic mass units). To separate all these masses requires an instrument with a resolution of about 100 000. A double focusing technique is needed to meet resolutions of this order. The ions must first be resolved in respect of their velocity and then with respect to their mass. A double focusing instrument is shown in Fig. 3. Ions formed by electron bombardment from the source are accelerated through a potential difference, as described earlier, and then through an electrostatic analyser. All ions emerging from this analyser

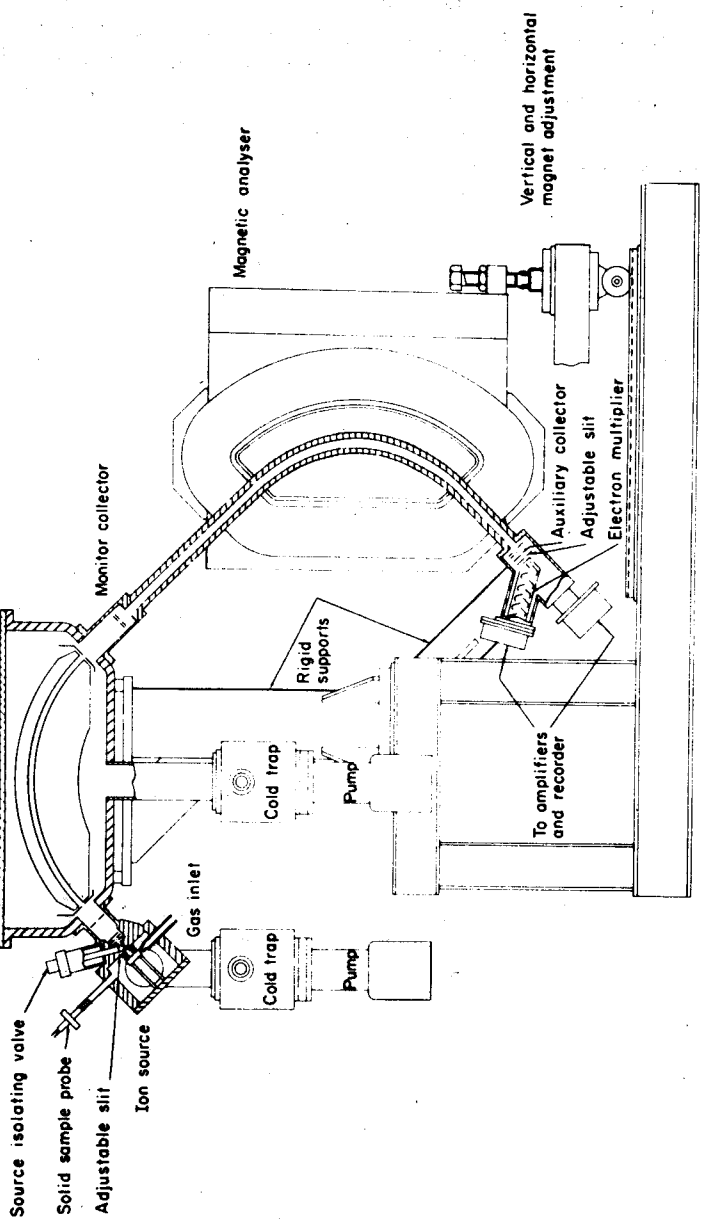

FIG. 3. The tube assembly for the double focusing mass spectrometer MS 9. (Published by kind permission of Kratos Ltd.)

have the same energy, there being a severe loss of intensity, of course, because all those ions with a slightly higher or slightly lower energy than the mean are extracted from the beam. In the second part of the instrument the ion beam is mass analysed in the conventional manner. In this instrument 90° has been chosen, an electro-magnet being used to generate the field.

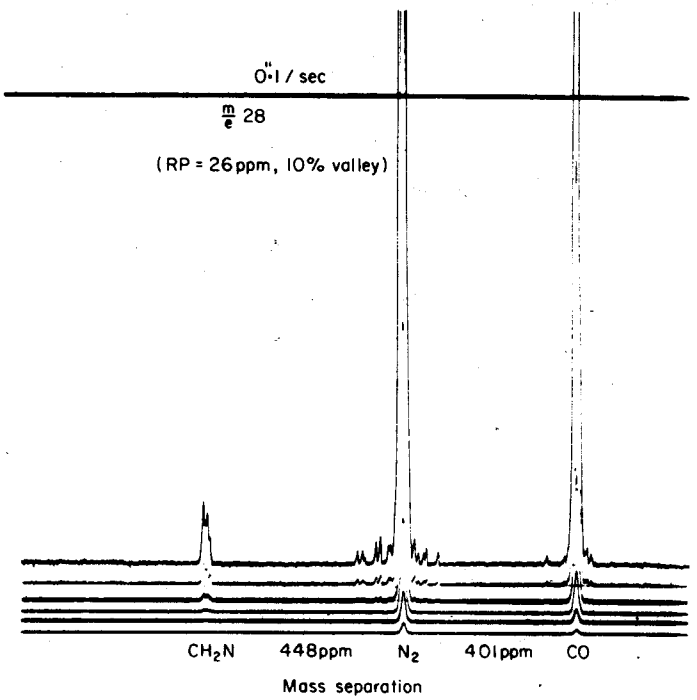

FIG. 4. Mass spectrum at 28 amu showing the separation of nitrogen and carbon monoxide in a double focusing instrument. (Published by kind permission of Kratos Ltd.)

In the double focusing instrument the path length is normally about 2 metres, the resolution being controlled by the width of the collimating slits. Because, for high resolution, these slits must be very narrow and because the initial ion beam intensity is reduced by the electrostatic analyser, the instruments usually need an electron multiplier to detect the very tiny currents reaching the collector. In practice the multiplier has a gain sufficiently high to allow the detection of individual ions.

Examples of the capabilities of double focusing instruments are shown in Figs 4 and 5. The two large peaks in Fig. 4 correspond to nitrogen and

carbon monoxide, both having a nominal mass of 28 amu. These are difficult to separate on a single focusing instrument. In Fig. 5 the peak on the left occurs at 211.07280 amu and the one on the right at 211.07436 amu. The difference in mass between these two peaks is about 0.001 amu. The machine described in Fig. 4, having a resolution of about 140 000, is adequate to differentiate between the two peaks. (This is an important example from chemical analysis as it differentiates between two

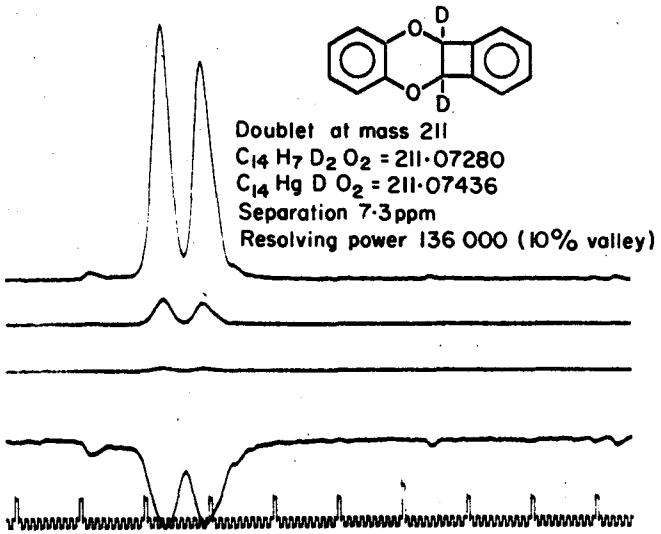

FIG. 5. A portion of the spectrum from a high resolution, double focusing mass spectrometer (MS 50), showing a doublet at mass 211. (Published by kind permission of Krato Ltd.)

atoms, one of which has a deuterium tag. The peak at the lower mass is from a molecule with two hydrogen atoms; the one at the higher mass is from a molecule in which one of the hydrogen atoms has been replaced by a deuterium atom, the other lost in the ionization process.)

Fortunately much of practical mass spectrometry does not require the expensive double focusing technique. For many applications, particularly when using instruments in connection with gas chromatography (GC/MS), a resolving power of the order of 1000 is quite adequate. The use of such modest resolving powers is possible because the bombardment of simple molecules by the electron beam in the ion source causes all large molecules to fragment, producing characteristic arrays of peaks in the mass spectrum. The mass spectrum of any given large molecule is, in fact, a "fingerprint". An example for a particular pesticide is shown in Fig. 6(a). The parent