

Back to Basics Section A: Ionization Processes

CHAPTER A1

CHEMICAL IONIZATION (CI)

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Quick Guide

- This article should be read in conjunction with that entitled, 'Electron Ionization'.
- In high vacuum (low pressure; 10^{-6} mbar) molecules and electrons interact to form ions (electron ionization). These ions are usually injected into the mass spectrometer analyser section.
- Still under vacuum but at higher pressure (typically 10^{-3} mbar), the initially formed ions collide with neutral molecules to give different kinds of ions before they are injected into the analyser.
- As an example, at low pressure, methane gas (CH_4) is ionized to give molecular ions ($\text{CH}_4^{\bullet+}$) but, at higher pressures, these ions collide with other CH_4 molecules to give carbonium ions (CH_5^+).
- If there is a substance (M) present which is under investigation, its molecules can collide with CH_5^+ with transfer of a proton (H^+) to give $[\text{M}+\text{H}]^+$, which may be called quasimolecular or protonated molecular ions.
- Because this chemical reaction occurs between the CH_5^+ and M species, the original methane (CH_4) is called a *reagent gas*, the CH_5^+ are reagent gas ions and the process is known as *chemical ionization (CI)*.
- There are other reagent gases, e.g. hydrogen (H_2), butane (C_4H_{10}), methanol (CH_3OH), ammonia (NH_3) and so on.
- The different reagent gases produce quasimolecular (protonated) ions ($[\text{M}+\text{H}]^+$) having more or less of an excess of internal energy, which may be enough to cause some fragmentation. Some reagent gases cause no decomposition (fragmentation) of $[\text{M}+\text{H}]^+$ ions but others do to some extent.
- Other reactions between reagent gas ions and molecules (M) can occur. As examples, ions $[\text{M}+\text{X}]^+$, can be formed, where X is Na, NO or NH_3 .
- Mass spectra from chemical ionization are not the same as those from electron ionization (EI).
- Mass spectra from EI usually contain many fragment ions and, often, not many molecular ions ($\text{M}^{\bullet+}$). Mass spectra from CI contain few fragment ions and, usually, lots of quasimolecular ions ($[\text{M}+\text{X}]^+$).
- Molecular ions ($\text{M}^{\bullet+}$) from EI have the same relative molecular mass (molecular weight) as the original molecules (M) but quasimolecular ions ($[\text{M}+\text{X}]^+$) have a different mass from that of the original molecules.

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- In similar ways, negative ions $[M-H]^-$ or $[M+X]^-$ can be formed by using a negative reagent gas.
- EI and CI are complementary techniques and are usually combined into one ion source so that rapid switching between the two can take place.

Summary Chemical ionization produces quasimolecular or protonated molecular ions which do not fragment as readily as the molecular ions formed by electron ionization. Therefore, CI spectra are normally simpler than EI spectra in containing abundant quasimolecular ions and few fragment ions. It is advantageous to run both CI and EI spectra on the same compound to obtain complementary information.

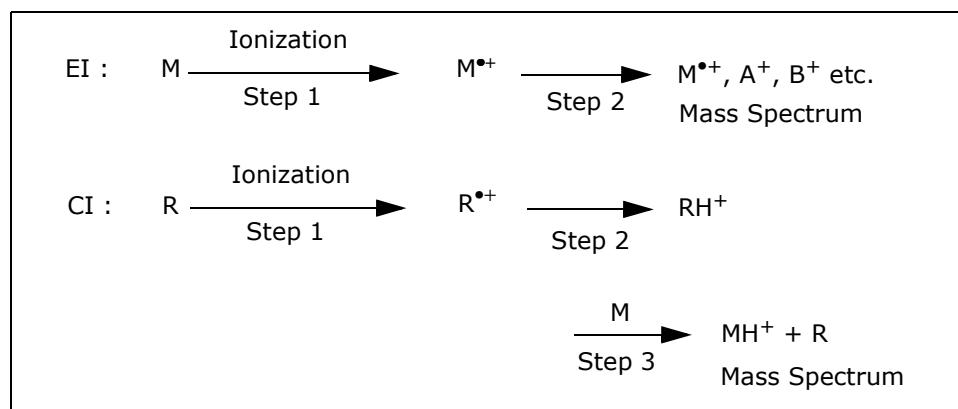


Figure I Comparison of basic EI and CI processes, showing different types of molecular ions and the formation of fragment ions in EI.

CHEMICAL IONIZATION

The Ionization Process

This section should be read in conjunction with the ‘Back-to-Basics’ article on ‘Electron Ionization’. In electron ionization (EI), high vacua (low pressures), typically 10^{-6} mbar, are maintained in the ion source so that any molecular ions ($M^{•+}$) formed initially from the interaction of an electron beam and molecules (M) do not collide with any other molecules *before* being expelled from the ion source into the mass spectrometer analyser (see: ‘Ion Optics’).

Decomposition (fragmentation) of such molecular ions ($M^{•+}$) to form fragment ions (A^+ , B^+ , etc.) occurs mostly in the ion source and the assembly of ions ($M^{•+}$, A^+ , B^+ , etc.) is injected into the mass spectrometer mass analyser. For chemical ionization (CI), the initial ionization step is the same as in EI but the subsequent steps are different (Figure 1). For CI, the gas pressure in the ion source is increased to, typically, 10^{-3} mbar, and sometimes up to atmospheric pressure, by injecting a reagent gas (R in Figure 1).

The substance being investigated (M) is present as only a small fraction of the reagent gas pressure. Thus, the electrons in the electron beam mostly interact with the reagent gas to form reagent gas ions ($R^{•+}$) and not $M^{•+}$ ions. At the higher pressures, the initial reagent gas ions almost immediately suffer multiple collisions with neutral reagent gas molecules (R). During this process, new ions (RH^+) are produced (step 2, Figure 1); these ions are reagent gas ions.

Because lots of these are produced, there is a high probability that they will collide with sample molecules (M) and that a proton (H^+) will be exchanged to give protonated molecular ions ($[M+H]^+$) as shown (step 3, Figure 1). These (quasimolecular) ions contain little excess of internal energy following CI and therefore tend not to fragment. Whereas EI spectra contain peaks corresponding to both molecular and fragment ions, CI spectra are much simpler, mostly having only protonated molecular ion peaks. Negative reagent gases give abundant $[M-H]^-$ or $[M+X]^-$ ions.

An Example of the Chemical Ionization Process

The ion source, across which an electron beam passes, is filled with methane, the reagent gas. There is a high vacuum around the ion source and so, to maintain a high pressure in the source itself, as many holes as possible must be blocked off or made small. Interaction of methane (CH_4) with electrons (e^-) gives methane molecular ions ($CH_4^{•+}$) - see Figure 2a.

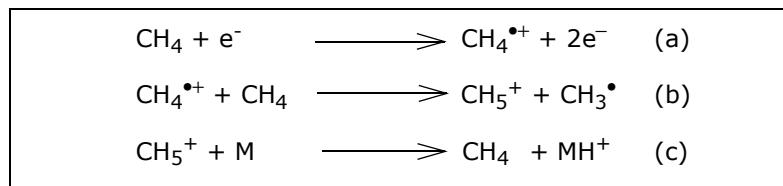


Figure 2 Formation of reactive ions (CH_5^+) from methane (CH_4) reagent gas and their reaction with sample molecules M to form protonated molecular ions MH^+ .

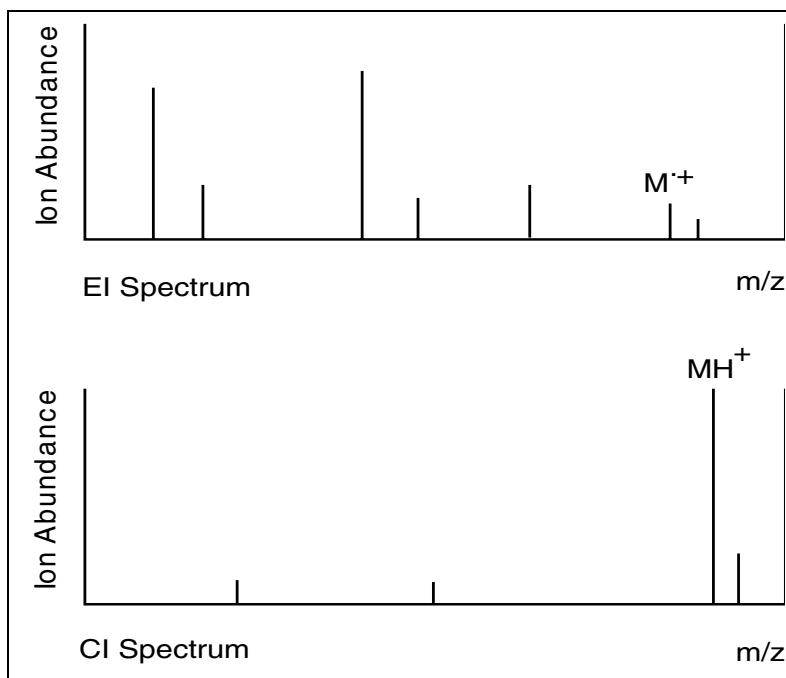


Figure 3 Comparison of EI and CI mass spectra illustrating the greater degree of fragmentation in the former and their greater abundance of quasimolecular ions in the latter.

Reagent gas	Molecular ion	Reactive reagent ion
H_2	$\text{H}_2^{\bullet+}$	H_3^+
C_4H_{10}	$\text{C}_4\text{H}_{10}^{\bullet+}$	$\text{C}_4\text{H}_{11}^+$
NH_3	$\text{NH}_3^{\bullet+}$	NH_4^+
CH_3OH	$\text{CH}_3\text{OH}^{\bullet+}$	CH_3OH_2^+
NO	$\text{NO}^{\bullet+}$	NO^+

Figure 4 Some types of reagent gases and their reactive ions.

Newly formed ions ($\text{CH}_4^{\bullet+}$) collide several times with neutral molecules (CH_4) to give carbonium ions (CH_5^+) - Figure 2b. The substance (M) to be investigated is vaporized into the ion source where it collides with these ions (CH_5^+). Proton exchange occurs because, usually, organic substances (M) are stronger bases than is CH_4 and so protonated molecular ions (MH^+ or better $[\text{M}+\text{H}]^+$) are formed (Figure 2c). These ions are expelled from the ion source into the mass analyser of the mass spectrometer (see 'Ion Optics'). Protonated molecular ions are also called quasimolecular ions and may also be represented as $[\text{M}+\text{H}]^+$ rather than MH^+ .

Little or no fragmentation of $[\text{M}+\text{H}]^+$ ions occurs and so CI spectra are very simple compared with EI spectra. A comparison of the two is shown in Figure 3, where it can be seen that the EI spectrum gives few molecular ions ($\text{M}^{\bullet+}$) but many fragment ions (A^+ , B^+ , etc.) whereas the CI spectrum shows many (abundant) quasimolecular or protonated ions $[\text{M}+\text{H}]^+$ and few fragment ions.

Thus, the CI and EI spectra are complementary and there is often considerable advantage in obtaining both since EI gives structural information and CI confirms the relative molecular mass (molecular weight).

Other Reagent Gases

The example of methane as a reagent gas can now be extended to other species, some of which are shown in Figure 4, along with the principal ions formed. The various reagent gases do not all act in the same proton-exchange manner described above and some of the major variations are detailed below. However, all of the ionization effected by the reagent gases is characterized by its propensity to give spectra having very few fragment ions.

Negative reagent ions, such as Ce^- (from CH_2Ce_2), $\text{O}^{\bullet-}$ (from NO) and OH^- (from NO plus CH_4), react with molecules (M) either by abstracting a proton to give $[\text{M}-\text{H}]^-$ ions or by addition to form $[\text{M}+\text{X}]^-$ ions. Of these, OH^- is particularly useful because its high proton affinity leads to hydrogen abstraction from most classes of organic compounds (except alkanes) with little fragmentation. As with the EI/CI combinations, it is often convenient for structure elucidation to generate both positive and negative ion data by CI. Instruments are available, which can measure both positive and negative CI spectra simultaneously. For example a reagent gas mixture of CH_4 and NO gives two corresponding reagent gases, CH_5^+ and OH^- . Thus, both $[\text{M}+\text{H}]^+$ and $[\text{M}-\text{H}]^-$ quasimolecular ions can be formed from sample molecules (M). By alternately injecting

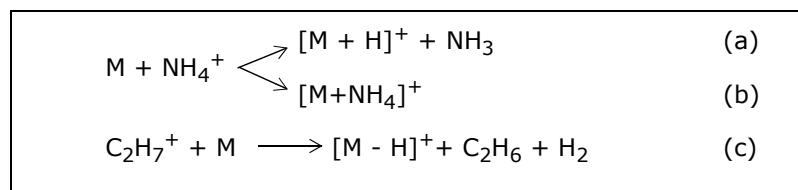


Figure 5 Typical CI processes in which neutral sample molecules (M) react with NH_4^+ to give either (a), a protonated ion ($[M + H]^+$) or (b), an adduct ion ($[M + NH_4]^+$); the quasimolecular ions are respectively 1 and 18 mass units greater than the true mass (M). In process (c), reagent ions ($C_2H_7^+$) abstract hydrogen, giving a quasimolecular ion, which is 1 mass unit less than M.

(pulsing) positive ions and negative ions (a process termed PPINICl) into the mass analyser (usually a quadrupole or ion trap), almost simultaneous positive and negative CI spectra can be measured.

Other Ionization Routes

Molecules of substrate (M) may not be ionized simply by the proton-transfer mechanism shown in Figure 2c. For example, with ammonia reagent gas, either $[M+H]^+$ ions or $[M+NH_4]^+$ ions can be formed, depending on the nature of the substrate (Figure 5). Process 5a is proton-exchange but Figure 5b shows an example in which the whole of the reagent gas ion attaches itself to the substrate to form a quasimolecular ion $[M+NH_4]^+$. In Figure 5a, the protonated molecular ion has a mass which is one unit greater than the true relative molecular mass since the mass of H is one. In Figure 5b, the adduct ion is greater than M by 18 mass units: (N=14, 4xH=4, total NH_4 =18).

If the substrate (M) is more basic than is NH_3 , then proton-transfer occurs but, if it is less basic, then addition of NH_4^+ occurs. Sometimes the basicity of M is such that both reactions occur and the mass spectrum contains ions corresponding to both $[M+H]^+$ and $[M+NH_4]^+$.

Sometimes, the reagent gas ions can form quasimolecular ions in which a proton has been removed from, not added to, the molecule (M) as shown in Figure 5c. In these cases, the quasimolecular ions have one mass unit less than the true molecular mass.

Uses of CI

Some substances under EI conditions fragment so readily that either no molecular ions survive or so few survive that it is difficult to be sure that the ones observed do not represent some impurity. Therefore, there is either no molecular mass information or it is uncertain. Under CI conditions, very little fragmentation occurs and, depending on the reagent gas, ions $[M + X]^+$ ($X = H, NH_4, NO$ etc.) or $[M-H]^+$ or $[M-H]^-$ or $[M+X]^-$ ($X=F, CL, OH, O$ etc) are the abundant quasimolecular ions, which do give molecular mass information.

Fragmentation under EI conditions yields structural information but CI yields little or none because it gives few fragment ions. Thus, CI is used mostly for molecular mass information and is frequently used with EI as a complement. Because there is little structural information, unlike for EI, there are no extensive libraries of CI spectra. CI spectra are apparent also in atmospheric pressure ionization systems (see 'Plasmaspray'). CI is often called a 'soft' ionization method because little excess of energy is put into the molecules (M) when they are

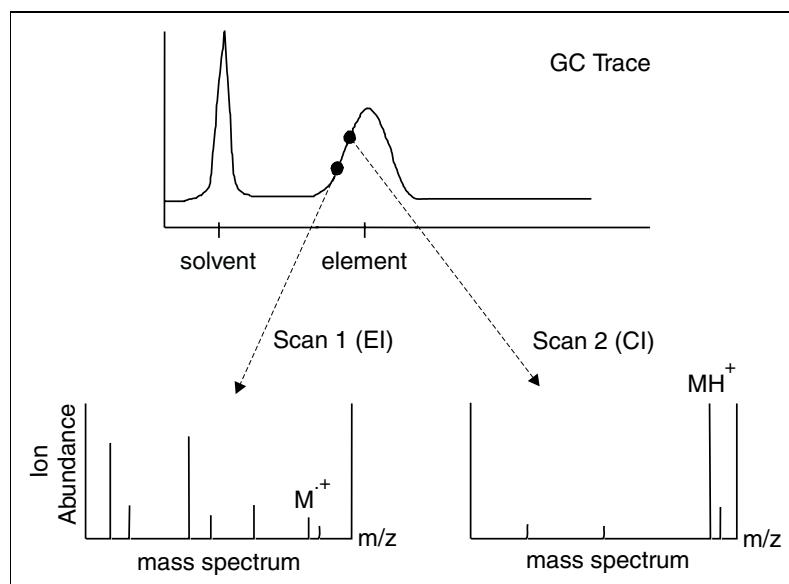


Figure 6 Successive spectra taken during elution of a substance from a GC column. The first spectrum obtained by EI shows many fragment ions whilst the second, obtained by CI, gives molecular mass information.

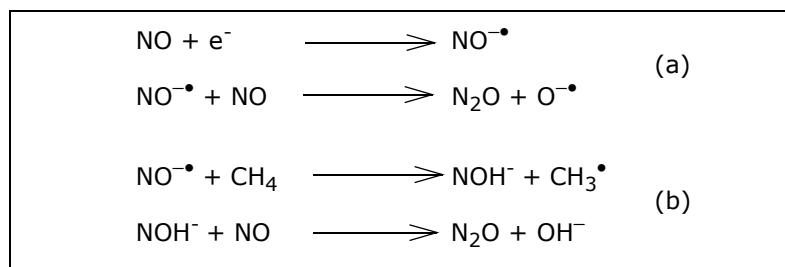


Figure 7 Negative reactant gas ions, O^{\bullet} and OH^- may be produced easily from (a) NO and (b) NO/CH_4 respectively.

ionized. Therefore, substances which might not otherwise give mass spectra containing molecular ions will give molecular mass information under CI conditions.

Use of CI/EI in Tandem in GC-MS

As shown above, CI and EI spectra complement each other and they are used frequently in such techniques as GC-MS where successive mass spectral scans are recorded. Alternate scans can be arranged to be either EI or CI, by alternate evacuation of reagent gas from or pressurisation with reagent gas into the ion source through which the GC effluent is flowing. With modern pumping systems, this switchover is complete within a few seconds. Figure 6 illustrates the EI/CE switching process and the sort of information obtained.

Negative Ions

Analogous to the formation of protonated ions, negative ions can be formed by deprotonation (Figure 7). Reagent gas ions such as OH⁻ or O^{•-} are strongly basic and capable of abstracting a proton from sample molecules M to give [M - H]⁻ ions, one mass unit less than the true molecular mass. Negative ion CI is a useful sensitive technique for substances having a high electron affinity, such as halo compounds and polycyclic aromatic hydrocarbons.

Conclusion

At higher pressures (~10⁻³ mbar) reagent gases such as methane, butane, ammonia and so on form reagent ions (CH₅⁺, C₄H₁₁⁺, NH₄⁺, etc.). These reagent ions react with sample molecules (M) to give stable [M+X]⁺ ions (X = H, NH₄, etc.) or [M - H]⁺ ions. Negative ions [M-H]⁻ may be formed by using a negatively charged reactant gas species. These ions have relative molecular masses different from that of the molecule (M) and are called quasimolecular ions. Negative ions may be obtained from negative reagent gases such as Cl⁻ or OH⁻. The CI technique is particularly useful for providing molecular mass information since little or no fragmentation occurs, unlike with EI. Ion sources for CI usually operate under either CI or EI conditions with fast switching between modes so that alternate CI, EI spectra can be recorded.

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CHAPTER A2

LASER DESORPTION IONIZATION AND MALDI

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Quick Guide

- A laser is a device for producing ultraviolet, visible or infrared light of a definite wavelength unlike most other light sources, which give out radiation over a range of wavelengths. The output of a single wavelength of light is described as being coherent.
- Lasers may be tuneable, viz., although only one wavelength is emitted at any one setting, the actual wavelength can be varied over a small range by changing the setting of the laser.
- Other notable characteristics of the laser are concerned with the intensity of the light emitted, its pulsed nature and the fine focusing that is possible.
- For many lasers used in scientific work, the light is emitted in a short pulse, lasting only a few nanoseconds but the pulses can be repeated at very short intervals. Other lasers produce a continuous output of light.
- The emitted beam of coherent radiation is narrow and can be focused into a very small area. This means that the density of radiation that can be delivered for any one pulse over a small area is very high, much higher than can be delivered by conventional light sources operating with similar power inputs.
- If the target at which a laser beam is directed can absorb light of the laser wavelength then the target will absorb a large amount of energy in a very small space in a very short time.
- The absorption of so much energy by a small number of target molecules in such a short time means that their internal energy is greatly increased rapidly and the normal processes of energy dissipation (such as heat transfer) do not have time to occur. Much of this excess of energy is converted into kinetic energy so that the target molecules are vaporized (ablated) and leave the target zone.
- Some of the target molecules gain so much excess of internal energy in a short space of time that they lose an electron and become ions. These are the molecular cation-radicals found in mass spectrometry by the direct absorption of radiation. However, these initial ions may react with accompanying neutral molecules, as in chemical ionization, to produce protonated molecules.

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- The above direct process does not produce a high yield of ions but it does form a lot of molecules in the vapour phase. The yield of ions can be greatly increased by applying a second ionization method (e.g., electron ionization) to the vaporized molecules. Therefore, laser desorption is often used in conjunction with a second ionization step, such as electron ionization, chemical ionization or even a second laser ionization pulse.
- Laser desorption is particularly good for producing ions from analytically 'difficult' materials. For example, they may be used with bone, ceramics, high molecular mass natural and synthetic polymers and rock or metal specimens. Generally, few fragment ions are formed.
- Improved ionization may be obtained in many cases by including the sample to be investigated in a matrix formed from sinapic acid, nicotinic acid or other materials. This variant of laser desorption is known as matrix-assisted laser desorption ionization (MALDI).
- The laser may be used as a finely focused beam, which with each pulse, drills deeper and deeper into the specimen giving 'depth profiling'. Alternatively, the beam can be defocused and moved over an area at lower power so as to explore only surface features of a specimen.

Summary

Lasers are used to deliver a focused high density of monochromatic radiation to a sample target, which is vaporized and ionized. The ions are detected in the usual way by any suitable mass spectrometer to produce a mass spectrum. The yield of ions is often increased by using a secondary ion source or a matrix.

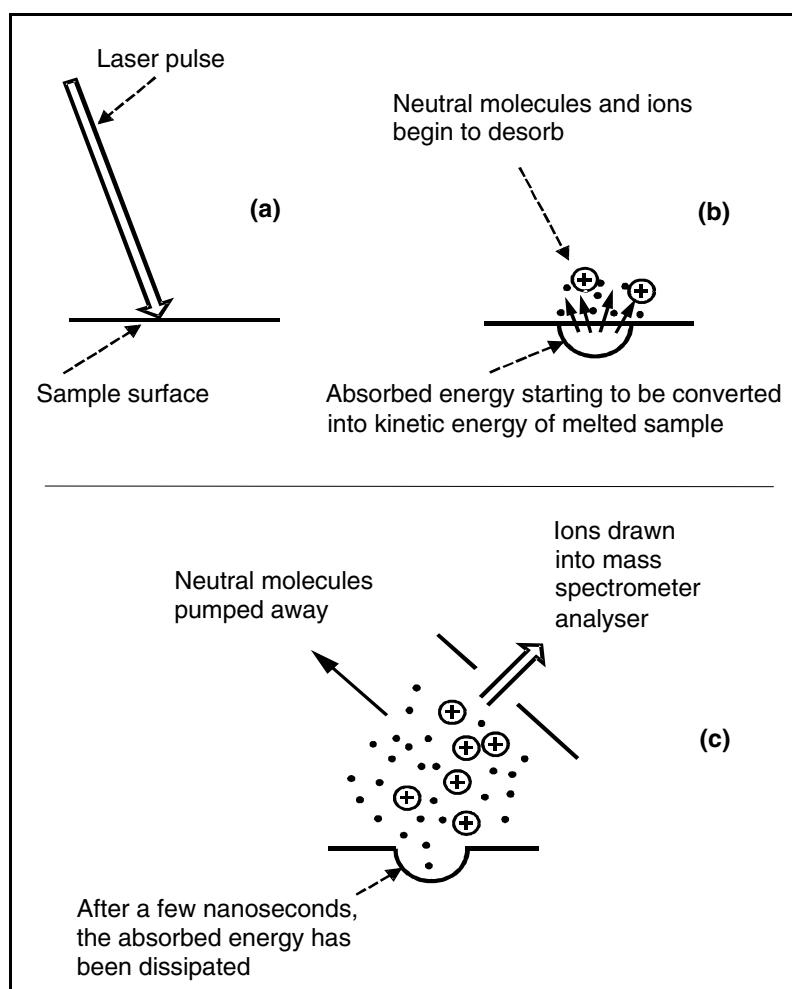


Figure I A laser pulse strikes the surface of a sample (a), depositing energy which leads to melting and vaporization of neutral molecules and ions from a small confined area (b). A few nanoseconds after the pulse, the vaporized material is either pumped away or, if it is ionic, it is drawn off into the analyser of a mass spectrometer (c).

LASER DESORPTION IONIZATION

The Ionization Process

A molecule naturally possesses rotational, vibrational and electronic energy. If it is a liquid or a gas, it will also have kinetic energy of motion. Under many everyday circumstances, if a molecule or group of molecules have their internal energy increased (e.g., by heat or radiation) over a *relatively* long period of time (which may only be a few microseconds), the molecules can equilibrate the energy individually and together so that the excess of energy is dissipated to the surroundings without causing any change in molecular structure. Beyond a certain point of too much energy in too short a time, the energy cannot be dissipated fast enough so that the substance melts and then vaporizes as internal energy of vibration and rotation is turned into translational energy (kinetic energy or energy of motion); simultaneous electronic excitation may be sufficient that electrons may be ejected from molecules to give ions. Thus, putting a lot of energy into a molecular system in a very short space of time can cause melting, vaporization, possible destruction of material and, importantly for mass spectrometry, ionization (Figure 1).

A laser is a device that can deliver a large density of energy into a small space. The *actual* energy given out by a laser is normally relatively small but, as it is focused into a very tiny area of material, the energy delivered per unit area is very large. The analogy may be drawn of sunlight which, although representing a lot of light, will not normally cause an object to heat up so that it burns. However, if the sunlight is focused into a small area by means of a lens, it becomes easy to set an object on fire or to vaporize it. Thus, a low total output of light radiation concentrated into a tiny area actually gives a high density or flux of radiation (we could even say a high light 'pressure') - this is typical of a laser. As an example, a Nd-YAG laser operating at 266 nm can deliver a power output of about 10 Watts, somewhat like a side-light on a motor car. However, this energy is delivered into an area of about 10^{-7} cm^2 so that the power focused onto the small irradiated area is about $10/10^{-7} = 10^8 \text{ Watts/cm}^2 = 10^5 \text{ Kilowatts/cm}^2$ (the same effect as focusing the heat energy from 100,000 'one bar' electric fires onto the end of your finger!)

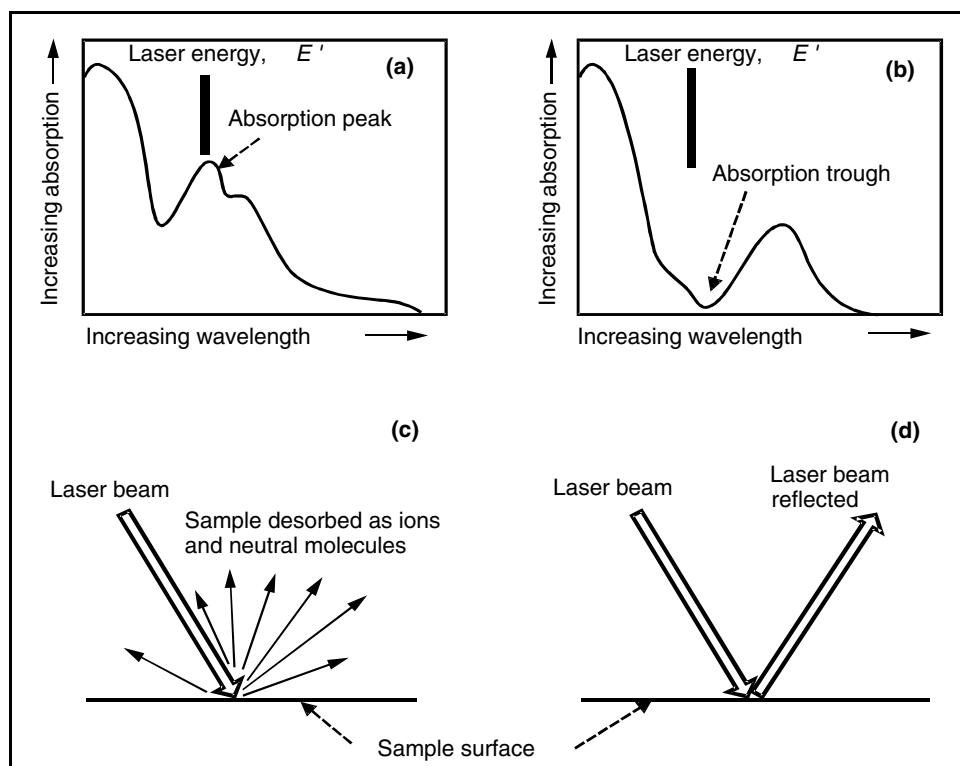


Figure 2 In (a), a pulse of laser light of a specific wavelength of energy, E' , strikes the surface of a specimen which has a light absorption spectrum with an absorption peak near to the laser wavelength. The energy is absorbed, leading to the ablation of neutral molecules and ions (c).
 In (b), the laser strikes the surface of a specimen that does not have a corresponding absorption peak in its absorption spectrum. The energy is not absorbed but is simply reflected or scattered (d), depending on whether the surface is smooth or rough.

No wonder sample molecules get agitated by the laser, even if it is only a few of them that are affected because of the small area which is irradiated).

A molecular system exposed to a laser pulse (or beam) has its internal energy vastly increased in a very short space of time, leading to *melting* (with increased rotational and vibrational and electronic energy), *vaporization* (desorption; increased kinetic or translational energy), some *ionization* (electronic excitation energy leading to ejection of an electron) and possibly some *decomposition* (increase in total energy sufficient to cause bond breaking). If enough energy is deposited into a sample in a very short space of time, it has no time to dissipate the energy to its surroundings and it is simply blasted away from the target area because of a large gain in kinetic energy (the material is said to be *ablated*). Laser desorption ionization is the process of beaming laser light, continuously or in pulses, onto a small area of a sample specimen so as to desorb ions, which are examined in the usual way by a mass spectrometer.

With continuous lasers (for example an argon ion laser), the energy delivered is usually much less than from pulsed ones and the focusing is not so acute. Thus, the irradiated area of the sample is more like 10^{-4} cm² rather than 10^{-7} cm² and the energy input is much less at about 100 Kilowatts/cm² rather than the 100,000 Kilowatts/cm² described above.

Other Considerations on Laser Desorption Ionization

Consider a laser emitting radiation of energy, E' . For a substance to absorb that energy, it must have an absorption spectrum (ultraviolet, visible or infrared) that matches that energy. Figure 2 shows two cases, one (a), in which a substance can absorb the energy, E' , and one (b), in which it cannot absorb this energy. In the second case, since energy cannot be absorbed, the laser radiation is reflected and none of its energy is absorbed. In the second case, much or all of the available energy can be absorbed and must then be dissipated somehow by the system. This dissipation leads to the effects itemized above. It follows that the capacity of a laser to desorb or ionize a substance will depend on three factors, one the actual wavelength (energy, E') of the laser light, two the power of the laser and three, the absorption spectrum of the substance being irradiated.

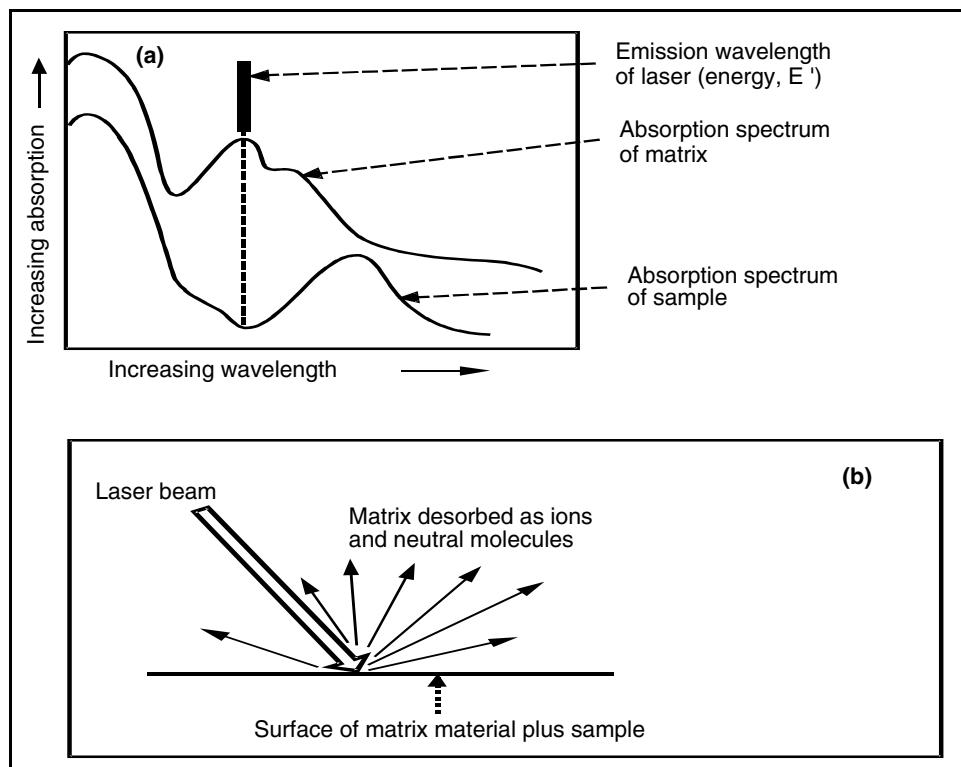


Figure 3 In a MALDI experiment, the sample is mixed or dissolved in a matrix material, which has an absorption spectrum that matches the laser wave length of energy, E' . The sample may not have a matching absorption peak (a) but this is not important because the matrix material absorbs the radiation, some of which is passed on to the dissolved sample. Neutral molecules and ions from both sample and matrix material are desorbed (b).

When the first and third factors match most closely and a lot of power is available (large light flux), a lot of the laser energy can be absorbed by the substance being examined; when the first and third factors mismatch, whatever the power, little or none of the laser energy is absorbed. Therefore, for any one laser wavelength, there will be a range of responses for different substances and, for this reason, it is often advantageous to use a *tunable* laser so that various wavelengths of irradiation can be selected to suit the substance being examined.

Use of a Matrix

There is another way of allowing for the above variability of ionization during laser irradiation. Suppose there is a sample substance (a matrix material) having an absorption band that matches closely the energy of the laser radiation. On irradiating this material, it will be rapidly increased in energy and will desorb and ionize quickly, as described above. Now suppose that it is not just the matrix material alone but is a mixture or solution (a matrix) of a substance to be examined with the matrix material. Now, at least some of the energy absorbed by the matrix can be passed on to the sample substance causing it to desorb and ionize (Figure 3a,b). This technique depends on the laser energy matching an absorption band in the matrix and a match with the sample substance is unnecessary so that the method becomes general. It is called, matrix-assisted laser desorption ionization (MALDI). Commonly, sinapic acid (3,5-dimethoxy-4-hydroxycinnamic acid) or nicotinic acid are used as matrix materials for examining organic and other compounds. The ions produced are usually protonated molecules, $[M + H]^+$, with few fragment ions.

Types of Laser

In theory, any laser can be used to effect desorption and ionization provided it supplies enough energy of the right wavelength in a short space of time to a sample substance. In practice, for practical reasons, the lasers, which are used tend to be restricted to a few types. The laser radiation can be pulsed or continuous (*continuous wave*). Typically, laser energies corresponding to the ultraviolet or near visible region of the electromagnetic spectrum (e.g., 266 or 355 nm) or the far infrared (about 20 mm) are used. The lasers are often tuneable over a range of energies.

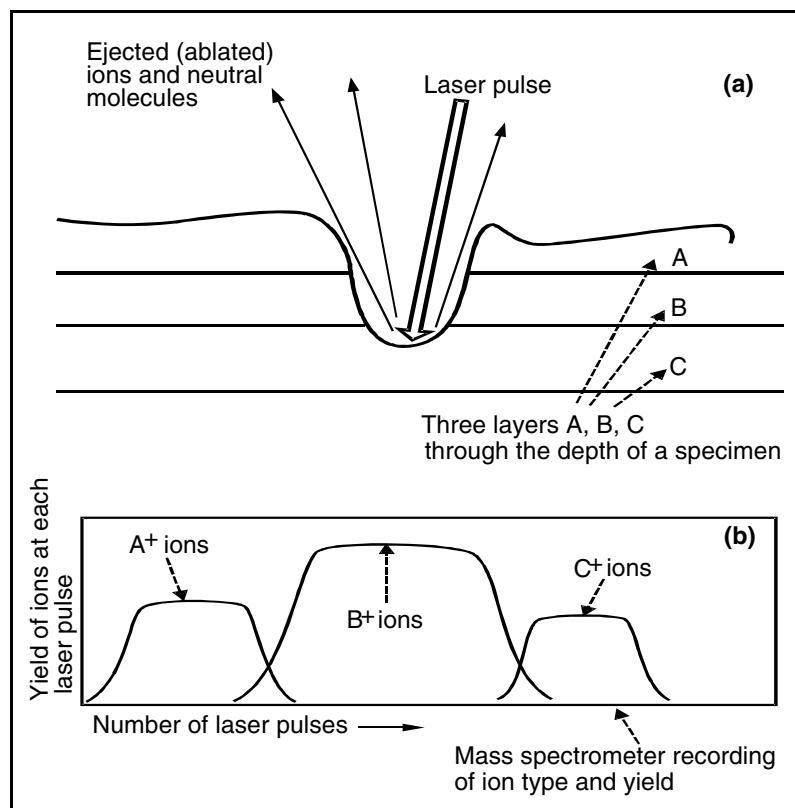


Figure 4a & b A laser pulse strikes the surface of a specimen (a), removing material from the first layer, A. The mass spectrometer records the formation of A^+ ions (b). As the laser pulses ablate more material, eventually the layer, B, is reached, at which stage, A^+ ions begin to decrease in abundance and B^+ ions appear instead. The process is repeated when the B/C boundary is reached so that B^+ ions disappear from the spectrum and C^+ ions appear instead. This method is very useful for depth profiling through a specimen, very little of which is needed.

The so-called ‘peak’ power delivered by a pulsed laser is often far greater than that for a continuous one. Whereas many substances absorb radiation in the ultraviolet and infrared regions of the electromagnetic spectrum, relatively few substances are coloured. Therefore, a laser which emits only visible light will not be so generally useful as ones emitting in the ultraviolet or infrared ends of the spectrum. Further, with a ‘visible’ band laser, coloured substances absorb more or less energy depending on the colour. Thus, two identical polymer samples, one dyed red and one blue, would desorb and ionize with very different efficiencies.

Secondary Ionization

Much of the energy deposited in a sample by a laser pulse or beam desorbs neutral material and not ions. Ordinarily, the neutral substances are simply pumped away and the ions are analysed by the mass spectrometer. To increase the number of ions formed, there is often a second ion source to produce ions from the neutral materials, thereby enhancing the total ion yield. This secondary or additional mode of ionization may be effected by electrons (electron ionization, EI), reagent gases (chemical ionization, CI) or even a second laser pulse. The additional ionization is usually organized as a pulse (electrons, reagent gas or laser), which follows very shortly after the initial laser desorption.

Uses of Lasers

Laser desorption methods are particularly useful for substances of high mass such as natural and synthetic polymers. Glycosides, proteins, large peptides, enzymes, paints, ceramics, bone and large polymers are all amenable to laser desorption mass spectrometry, with the sample being examined either alone or as part of a prepared matrix. Because of the large masses involved, for pulsed laser desorption, the method is frequently used with time-of-flight or ion trap instruments, which need pulses of ions. For MALDI, sample preparation can be crucial, the number of ions produced varying greatly with both the type of matrix material and with the presence of impurities. Fragment ions are few but the true molecular mass can be misinterpreted because of the formation of adduct ions between the matrix material and the substance under investigation; these adduct ions have greater mass than the true molecular mass. Some impurities, as with common ionic detergents, may act as suppressants to ion formation.

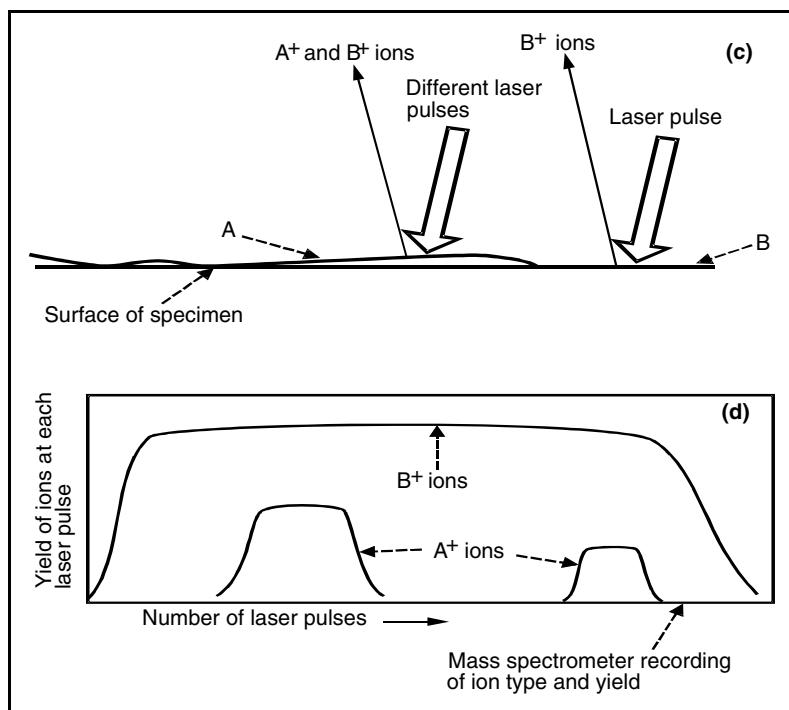


Figure 4c & d In (c), less power is used and the laser beam is directed at different spots across a specimen. Where there is no surface contamination only B^+ ions appear but, where there is surface impurity then ions A^+ from the impurity also appear in the spectrum (d).

The laser approach without a matrix can be employed in two main ways. Since the intensity and spot size of the laser pulse or beam can be adjusted, the energy deposited into a sample may range from a very large amount confined to a small area of sample to much less spread over a larger area. Thus, in one mode, the laser can be used to penetrate down through a sample, each pulse making the previously ablated depression deeper and deeper. This is *depth profiling*, which is useful for examining the variation in composition of a sample with depth (Figure 4a). For example, gold plating on ceramic would show only gold ions for the first laser shots until a hole had been drilled right through the gold layer; there would then appear ions such as sodium and silicon that are characteristic of the ceramic material and the gold ions would mostly disappear.

By using a laser with less power and the beam spread over a larger area, it is possible to sample a surface. In this approach, after each laser shot, the laser is directed onto a new area of surface, giving *surface profiling* (Figure 4c). At the low power used, only the top few nanometers of surface are removed and the method is suited to investigation of surface contamination. The normal surface yields characteristic ions but, where there are impurities on the surface, additional ions appear.

Laser desorption is commonly used for pyrolysis/mass spectrometry, in which small samples must be heated very rapidly to high temperatures to vaporize them before they are ionized. In this application of lasers, very small samples are used and the intention is not simply to vaporize intact molecules but also to cause ‘characteristic’ degradation (the Back-to-Basics guide on pyrolysis/mass spectrometry should be consulted).

Conclusion

Lasers may be used in either pulsed or continuous mode to desorb material from a sample, which may be examined as such or may be mixed or dissolved in a matrix. The desorbed (ablated) material contains relatively few or sometimes even no ions and a second ionization step is frequently needed to improve the yield of ions. Molecular or quasimolecular ions are mostly produced with few fragment ions. By adjusting the laser focusing and power, laser desorption can be used for either depth or surface profiling.

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Back to Basics Section A: Ionization Processes

CHAPTER A3

ELECTRON IONIZATION (EI)

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Quick Guide

- Molecules can interact with energetic electrons to give *ions* (electron ionization; EI) which are electrically charged entities. The interaction used to be called electron impact (also EI), although no actual collision occurs.
- Loss of an electron from a molecule (M) gives a (positive) *radical cation*, written $M^{\bullet+}$.
- Gain of an electron by a molecule gives a (negative) *radical anion* ($M^{\bullet-}$).
- The mass of an electron is very small and, for most practical purposes, the mass of an ion is the same as that of the corresponding neutral species, viz., $M \approx M^+ \approx M^-$.
- The interacting electrons are energized by acceleration in an electric field before they interact with molecules to give ions.
- Standard EI spectra are obtained with an electron energy of 70 volts (electrons accelerated through 70V).
- For most compounds, it is easier to produce positive ions than negative ones and most EI mass spectrometry is concerned with positive ions.
- Mass spectrometrically, the *mass-to-charge ratio* (m/z) is important. However, if $z = 1$, then conveniently, $m/z = m$.
- Removal of one (negative) electron from a molecule leaves a single positive charge, ($z = 1$). Loss of two electrons would give $z = 2$ and so on.
- For most EI mass spectrometry, $z = 1$, and higher charges can be neglected usually.
- The ion ($M^{\bullet+}$) derived from the parent molecule by loss of an electron is called a *molecular ion*.
- Depending on the structure of substance M and the energy of the incident electron, the resulting ion ($M^{\bullet+}$) may break up (fragment) to give ions of smaller mass (A^+ , B^+ , etc.).

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- A *mass spectrum* is a chart showing on the x-axis the mass of each ion (M_m , M_a , M_b etc.) and on the y-axis the number (abundance) of ions at each mass.
- The ion having greatest abundance is said to form the *base peak* in the spectrum. The base peak may or may not be the same as the molecular ion peak.

Summary

The interaction of electrons with molecules gives molecular ions, some of which can break down to give smaller fragment ions. The collection of molecular and fragment ions is separated by a mass analyser to give a chart relating ion mass and abundance (a mass spectrum).

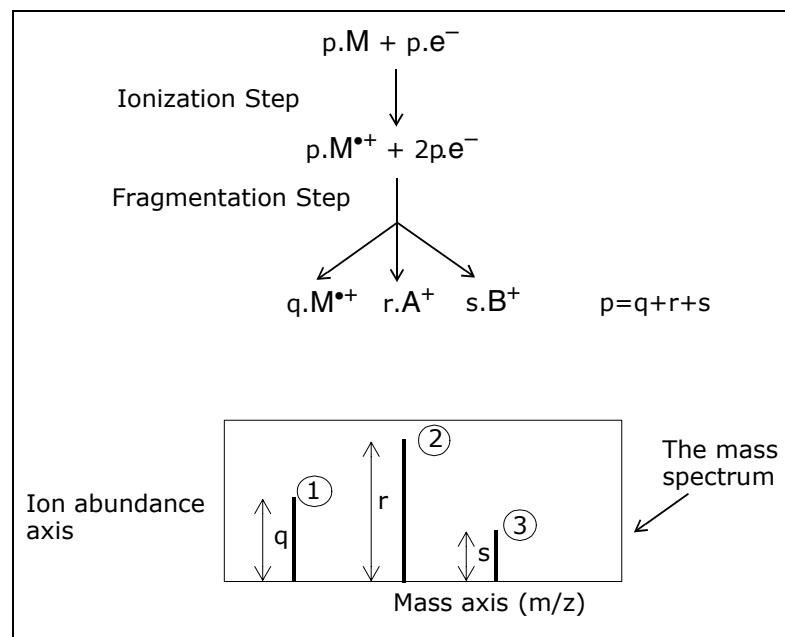
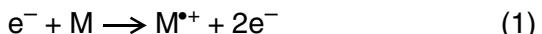


Figure I The formation of a simple EI mass spectrum from a number (p) of molecules (M) interacting with electrons (e^-). Peak 1 represents M^{*+} , the molecular ion, the ion of greatest mass (abundance q). Peaks 2, 3 represent A^+ , B^+ , two fragment ions (abundances r , s). Peak 2 is also the largest, and therefore the base peak.

ELECTRON IONIZATION (EI)

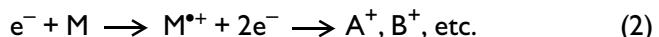
The Ionization Process

When an electron (negative charge) is accelerated through an electric field, it gains kinetic energy. Thus, after acceleration through 70 volts, the electron has an energy of 70 electron-volts (eV). These energetic electrons can interact with (uncharged, neutral) molecules (M), by passing close to or even through the molecule, so as to eject an electron from the molecule (equation 1).



Thus two electrons leave the reaction zone, leaving a positively charged species (M^{*+}) called an ion, (in this case, a *molecular ion*). Strictly, M^{*+} is a radical-cation. This electron/molecule interaction (or collision) was once called electron-impact (also EI), although no impact actually occurs.

At 70eV, in a high vacuum, the interaction between electrons and molecules leaves some ions (M^{*+}) with so much extra energy that they break up (fragment) to give ions of smaller mass (A^+ , B^+ etc.; equation 2).



This fragmentation is characteristic for a substance, similar to a fingerprint, and is referred to as a mass spectrum.

For a limited range of substances, negative radical-anions (M^{*-}) may be formed rather than positive ions (equation 3). Negative radical-anions may be produced in good abundance by methods other than electron ionisation. However, most EI mass spectrometry has been concerned with positive ions and only these are discussed here.



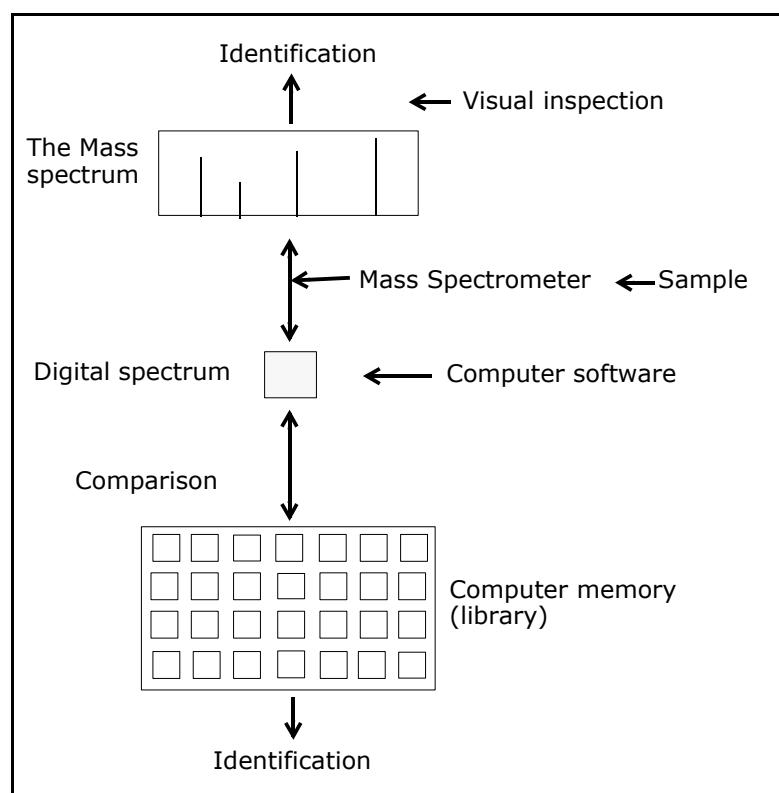


Figure 2 Visual and computer assisted identification of the structure of a sample analysed by a mass spectrometer.

**Mass-To-Charge Ratio
(m/z)**

The relative molecular mass of a molecule (M_r) is almost the same as that of the derived molecular ion (M^{*+}) because the mass of an electron is very small compared with the total mass of the molecule. For practical purposes in mass spectrometry, $M_r = M_r^{*+}$, and is written, M^{*+} .

An ion of mass (m) having a single positive charge ($z = 1$) has a *mass-to-charge ratio* of $m/z = m/1 = m$. Thus, the mass-to-charge ratio is conveniently equal to the mass of the ion and so $M^{*+}/z = M_r^{*+} = M_r$. Most ions produced by EI have a single charge and, as mass spectrometers utilize the charge to measure ion mass, it is fortunate that usually, $z = 1$. Similarly, the fragment ions (A^+ , B^+ , etc.) have single positive charges (Figure 1).

The Mass Spectrum

An EI mass spectrum is a chart on two axes relating the mass of an ion (m, or strictly m/z) to its abundance. During the ionization and fragmentation steps in the ion source (where electron/molecule reaction occurs) different numbers of ions (M^+ , A^+ , B^+ etc.) are produced and subsequently measured by the mass analyser. The numbers of individual ions are referred to as ion abundance. Thus, a mass spectrum records mass (or m/z) on the x-axis and corresponding ion abundance on the y-axis as a series of 'peaks'. The peak corresponding to the ion of greatest abundance is called the *base peak* which may correspond to the molecular ion or to any one of the fragment ions (Figure 1).

The mass spectrum is characteristic for different substances and can be used like a fingerprint, to identify a substance either by comparison with an already known spectrum or through skilled 'interpretation' of the spectrum itself (Figure 2).

The Ion Source

After ions have been formed by EI, they are examined for mass and abundance by the analyser part of the mass spectrometer which may incorporate magnetic sectors, electric sectors, quadrupoles, time-of-flight tubes and so on. The region in which the ions are first formed is called an *ion source*. The actual construction of (EI) ion sources varies considerably but all operate on the same principles.

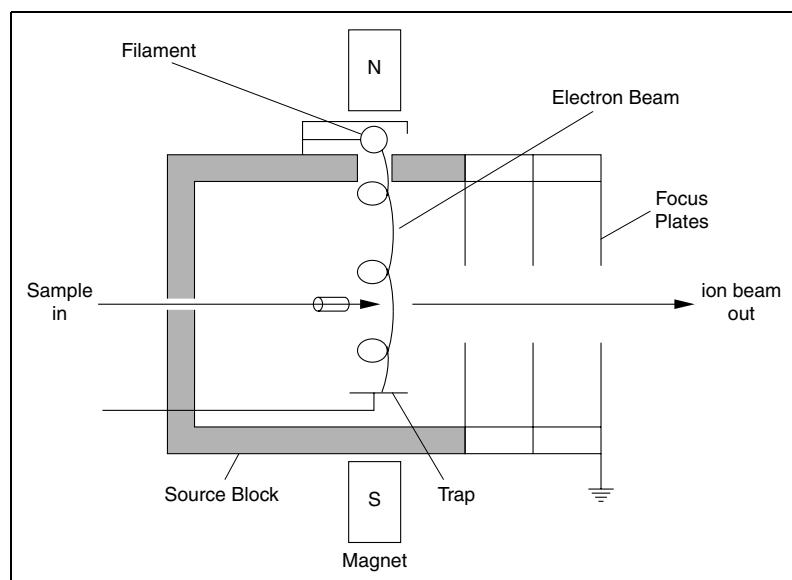


Figure 3 Simple ion source, showing the housing (block) with electron beam for EI.

The source can be regarded as a ‘box’ which is more or less gas-tight, depending on type and usage (Figure 3). Electrons are produced at one side of the ‘box’ (source) from a heated filament and are further energized by accelerating them through a potential of 70 volts. The moving electrons are directed across the ‘box’ and constrained into a ‘beam’ by a magnetic field. This *electron beam* interacts with sample molecules which have been vaporized into the source to give ions, as described above. The ions are extracted from the source by an electric field and passed into the analyser as an ion beam.

The presence and intensity of the electron beam are confirmed by allowing it to impinge on a ‘trap’ plate on the side of the ion source opposite to the heated filament.

Electron ionization occurs when an electron beam crosses an ion source ('box') and interacts with sample molecules, which have been vaporized into the source. Where the electrons and sample molecules interact, ions are formed, representing intact sample molecular ions and also fragments produced from them. These molecular and fragment ions compose the mass spectrum, which is a correlation of ion mass and its abundance. EI spectra of tens of thousands of substances have been recorded and form the basis of users' spectral libraries, either in book form or stored in computer-compatible memory banks.

Isotopes	A word of warning here. As described above, a simple compound such as methane (natural gas), which has a molecular formula of CH ₄ would be expected to give a mass of just 16 (C=12; H=1 C+4H=16). Only a peak at mass 16 in the spectrum would be expected. However, carbon occurs in a slightly ‘heavier’ variety as well (an isotope) in which C=13. Therefore, the mass spectrum will show two peaks, one at mass 16 and one at 17 (¹² CH ₄ = 16; ¹³ CH ₄ = 17). Because the two carbon isotopes exist in different amounts in nature, the peak heights at 16, 17 will not be equal. Figure 4a shows the mass spectrum for a compound with the isotope contributions included and Figure 4b shows the same spectrum, in which the ¹³ C isotope peaks have been removed. Clearly, one spectrum looks much more complex than the other. Other common elements such as hydrogen, oxygen, nitrogen, sulphur and so on also have isotopes, thereby complicating the appearance of a mass spectrum even more.
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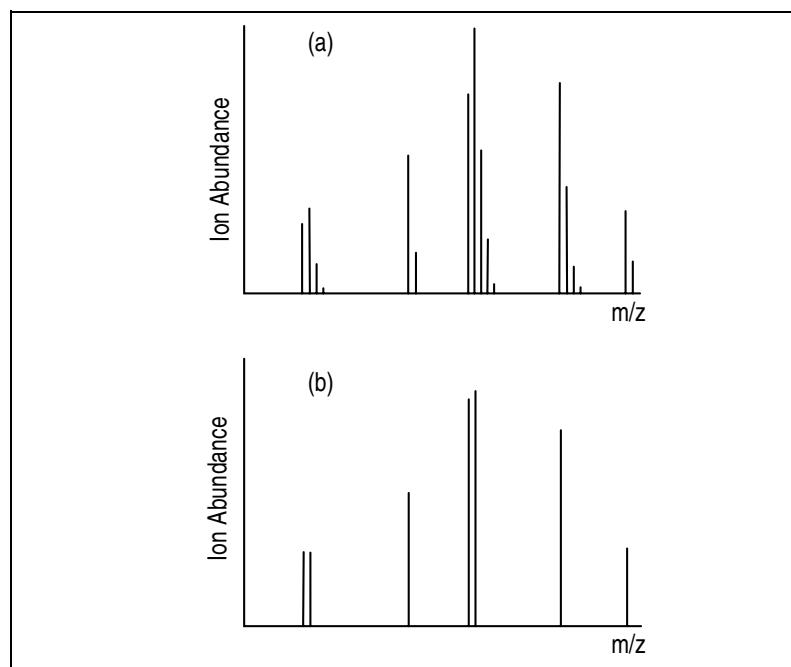


Figure 4 The mass spectrum of a carbon compound (a) with, and (b) without, the ^{13}C isotopes.

A significant number of elements have lots of isotopes and the presence of these correspondingly gives a spectrum having many peaks. On the other hand, the 'fingerprint' of these isotopes, gives mass spectrometry an important advantage in often being able to identify which elements are present simply from these fingerprint patterns.

A further important use of EI mass spectrometry lies in measuring isotope ratios, which can be used in estimating the ages of artefacts, rocks or fossils. Electron ionization affects the isotopes of any one element equally so that, in forming the mass spectrum, the true isotope ratio is not distorted by the ionization step. Back-to-Basics guides on Isotopes should be consulted for further information.

Conclusion	Molecules interact with electrons to give molecular and fragment ions, which are mass analysed. A mass spectrum relates the masses of these ions and their abundances.
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Back to Basics Section A: Ionization Processes

CHAPTER A4

FAB AND LSIMS IONIZATION

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Quick Guide

- In fast atom bombardment (FAB), an atom gun is used to project 'heavy' fast atoms (often argon or xenon) onto the surface of a target solution (matrix).
- The solution (or matrix) consists of the substance under investigation dissolved in a high boiling point solvent which does not evaporate quickly in the vacuum of a mass spectrometer.
- The impact of the fast atoms on the solution surface results in desorption of secondaries (positive ions, negative ions and neutrals) into the low pressure gas phase region above the matrix surface.
- By selecting either a large positive or negative voltage on a plate with a slit in it held above the surface, the desorbed negative or positive ions respectively can be accelerated away from the surface and into a mass analyser.
- The mass spectrometer provides a mass spectrum of the ions, coming from the matrix, some of which arise from the matrix itself and some from substances dissolved in it.
- Usually, FAB yields molecular or quasimolecular ions which have little excess of internal energy and therefore do not fragment. This ionization method is mild and good for obtaining molecular mass (molecular weight) information.
- Substrate molecules (M) usually give molecular radical-cations ($M^{\bullet+}$) or cationated molecular ions ($[M+X]^+$, $X = H, Na, K$ etc.) or negative ions. $[M-H]^-$.
- FAB has been used with a wide variety of substances, including thermally labile compounds such as peptides, sugars, carbohydrates and organometallics.
- Molecular masses up to about 2000 Daltons are fairly routine; above this value, the efficiency of the process drops off but is still useful up to about 4-5000 Daltons.
- Confusingly, FAB is sometimes called secondary ion mass spectrometry (SIMS), the 'secondary' referring to the nature of the process (primary bombardment, secondary emission), but see below.

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- Historically, the term SIMS was developed for bombardment of solid surfaces with ions and so, for greater descriptive precision, the name, liquid secondary ion mass spectrometry (LSIMS), is better and can be used synonymously with FAB.
- Instead of bombarding the matrix surface with fast atoms, fast ions can be used. Often these are caesium (Cs^+) ions.
- As with fast atoms, bombardment of the matrix with fast ions causes very similar desorption of ions and neutrals.
- In the case of fast ions, the terminology of secondary ion emission mass spectrometry (SIMS) is more obvious in that a primary incident beam of ions onto a target releases secondary ions after impact.
- Where the target is a liquid (matrix), the more descriptive term LSIMS should be used, as above.

Summary

The impact of a primary beam of fast atoms or ions on a target matrix (substrate and solvent) causes desorption of molecular or quasimolecular ions characteristic of the substrate. The process is called FAB for atom bombardment or LSIMS for ion bombardment.

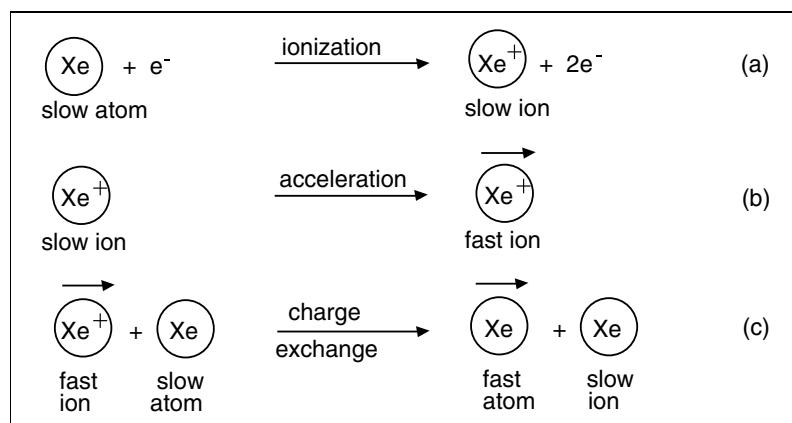


Figure I

- (a) Xenon atoms are ionized to Xe^+ , using electrons. These ions are relatively slow and move in all directions.
- (b) Xe^+ ions are accelerated through a high electric potential so that they attain a high speed in one direction.
- (c) Charge exchange between fast Xe^+ ions and slow Xe atoms gives a beam of fast Xe atoms and slow ions. The latter are removed by electric deflector plates, leaving just a beam of fast atoms.

FAB AND LSIMS IONIZATION

Introduction

It has long been known that bombardment of a solid surface by a primary beam of ions causes secondary ions characteristic of the solid target to be emitted. Examination of the spectrum of desorbed (secondary) ions led to the technique of secondary ion mass spectrometry (SIMS). This process was useful for some solids, particularly metals, but achieved no prominence in organic mass spectrometry because of surface charging (organics are usually insulators), the fleeting nature of the production of ions (if at all) and the extensive radiation damage caused to the target substance.

A big step forward came with the discovery that bombardment of a liquid target surface by a beam of fast atoms caused continuous desorption of ions, which were characteristic of the liquid. Where this liquid consisted of a sample substance dissolved in a solvent of low volatility (a matrix), both positive and negative molecular or quasimolecular ions characteristic of the sample were produced. The process quickly became known by the acronym FAB (fast atom bombardment) and for its then fabulous results on substances which had hitherto proved intractable. Later, it was found that a primary incident beam of fast ions could be used instead and a more generally descriptive term, LSIMS (liquid secondary ion mass spectrometry) has come into use. However, it may be noted that purists regard and refer to both FAB and LSIMS as simply facets of the original SIMS. In practice, any of the acronyms can be used but FAB and LSIMS are more descriptive when referring to the primary atom or ion beam.

When the liquid target is not a static pool but, rather, a continuous stream of liquid, the added description of 'dynamic' is used. Thus, dynamic FAB and LSIMS refer to bombardment of a continuously renewed (flowing) liquid target.

Atom or Ion Beams

A 'gun' is used to direct a beam of fast moving atoms or ions onto the liquid target (matrix). Figure 1 shows details of the operation of an atom gun. An inert gas is normally used for bombardment because it can produce no unwanted secondary species in the primary beam and contamination of the gun and mass spectrometer is avoided. Helium, argon and xenon have been used commonly but the higher mass atoms are preferred for maximum yield of secondary ions.

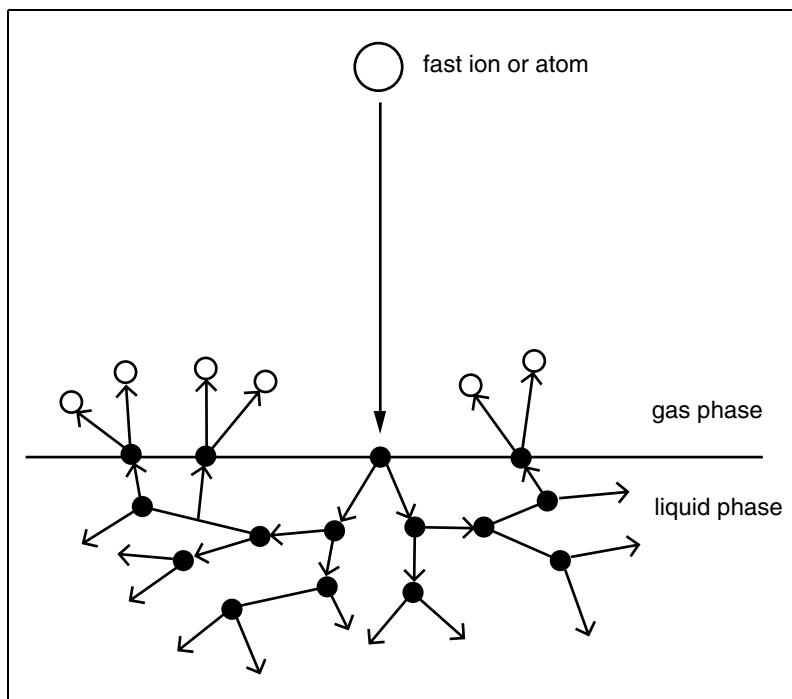


Figure 2 A typical cascade process. A fast atom or ion collides with surface molecules, sharing its momentum and causing the struck molecules to move faster. The resulting fast moving particles then strike others, setting up a cascade of collisions until all the initial momentum has been redistributed. The dots (●) indicate collision points. Ions or atoms (○) leave the surface.

In the gun, inert gas atoms are ionized to give positive ions which are immediately accelerated by an electric potential to give a high velocity beam of ions. As these ions collide with other inert gas atoms, charge exchange occurs such that many of the fast moving ions become fast moving atoms (Figure 1). Any residual ions are removed by an electric potential on a deflector plate, leaving a beam of fast moving atoms which exits from the 'gun'. The beam is somewhat divergent and the gun is situated near the target.

Instead of the fast atom beam, a primary ion beam gun can be used in just the same way. Generally, such an ion gun emits a stream of caesium ions (Cs^+) which are cheaper to use than xenon but still have large mass (atomic masses: Cs, 139; Xe, 131) and produce no fragment ions in the primary beam but they can contaminate the mass spectrometer with continued use.

The Ionization Process

When the incident beam of fast moving atoms or ions impinges onto the liquid target surface, major events occur within the first few nanometers, viz., momentum transfer, general degradation and ionization.

(i) Momentum transfer

The momentum of a fast moving atom or ion is dissipated by collision with the closely packed molecules of the liquid target. As each collision occurs, some of the initial momentum is transferred to substrate molecules, causing them in turn to move faster and strike other molecules. The result is a 'cascade' effect which results in some of the substrate molecules being ejected from the surface of the liquid (Figure 2). The process may be likened to throwing a heavy stone into a pool of water - some of the water splashes upwards.

Clearly, heavier and faster moving atoms or ions will cause more particles to be ejected from the surface through momentum transfer than will slower moving, lighter atoms or ions; this is the reason for preferring xenon or caesium.

If the liquid which is being bombarded already contains ions, then some of these will be ejected from the liquid and can be measured by the mass spectrometer. This is an important but not the only means by which ions appear in a FAB or LSIMS spectrum.

Momentum transfer of pre-formed ions in solution can be used to enhance ion yield, as by addition of acid to an amine to give an ammonium species (Figure 3).

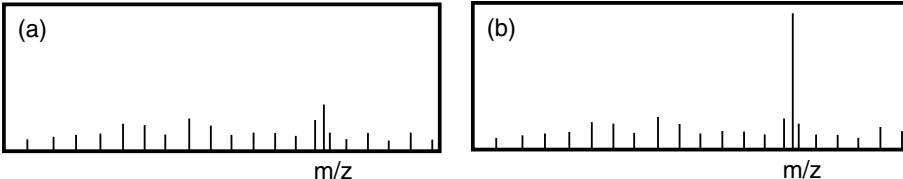
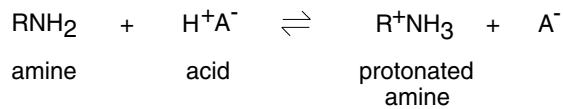


Figure 3 An example of enhanced ion production. The chemical equilibrium shown above exists in a solution of an amine (RNH_2). With little or no acid present, the equilibrium lies well to the left and there are few ‘pre-formed’ protonated amine molecules (ions, RNH_3^+); the FAB mass spectrum (a) is typical. With more or stronger acid, the equilibrium shifts to the right, producing more protonated amine molecules. Thus, addition of acid to a solution of an amine subjected to FAB, usually causes a large increase in the number of protonated amine species recorded (spectrum (b)).

(ii) Ionization by electron transfer

The close encounter of a fast moving atom or ion with a neutral molecule can lead to charge exchange in which an electron moves from one particle to another (Figure 4). In this way, positive and negative ions are formed separately from any pre-existing ions which might be present. Momentum transfer again leads to the newly formed ions being ejected from the liquid.

(iii) Random fragmentation

As well as the two specific effects discussed in (i) and (ii), bombardment of an organic liquid (solution) with a stream of atoms or ions having very high kinetic energies leads to fairly indiscriminate (random) bond cleavages to produce radicals and small neutral substances which, in turn, can be further fragmented or can recombine to form other neutrals. All of these 'bits' of fragmented or synthesized molecules can be ionized also. Therefore, an FAB or LSIMS spectrum will have a background of peaks at almost every m/z value and of fairly uniform height. The appearance is rather like viewing grassland by lying down and looking along or through the grass. Indeed, the FAB background is sometimes called 'grass'.

A major advantage of using a liquid target in SIMS lies in the fact that these randomly produced fragments appear first of all in the surface layers and then diffuse more or less rapidly into the bulk of the liquid, i.e., they are greatly diluted as new surface is constantly formed. On the other hand, sample molecules are distributed throughout the liquid at the start and, as they disappear from the surface during bombardment, they are continually replaced.

Thus, the liquid (matrix) reservoir disperses and dilutes the fragments which would otherwise give a large background of ions and provides a flow of new sample (and solvent) molecules to the surface so that molecular and quasimolecular ions are continuously replenished.

Despite the high kinetic energy of the bombarding atoms or ions and transfer of some of this energy during collision, the newly formed ions collide with other molecules and, before being ejected from the surface of the liquid, lose most of any excess of internal vibrational and rotational energy. Therefore, these ions do not fragment and so any sample substance dissolved in the liquid shows up as molecular or quasimolecular positive or negative ions.

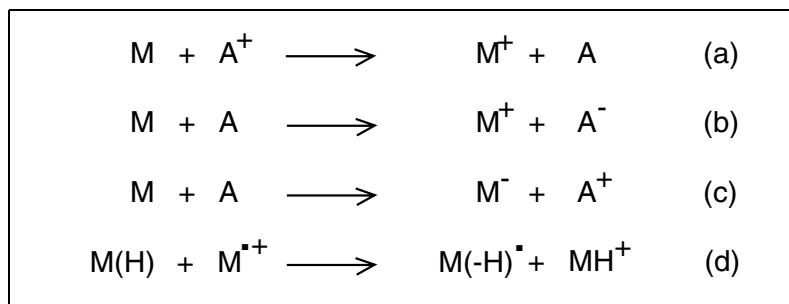


Figure 4 Collision of a fast moving ion (A^+) or atom (A) with neutral molecules (M) can lead to an electron being stripped from the molecule by charge exchange to give an ion (M^+) or can lead to an electron being deposited in the molecule to give M^- (processes a, b, c). The initially formed ion (M^+) can remove a proton from another molecule, $M(H)$, to give protonated molecular ions, MH^+ (process d).

The ionization process is mild, with no additional heat being necessary for vaporization. Therefore thermally labile molecules like peptides are readily amenable to FAB or LSIMS, giving good molecular mass information.

Properties of the Solvent (Matrix)

Liquids examined by FAB or LSIMS are introduced on the end of a probe until the liquid sits in the atom or ion beam. Because of the high vacuum conditions existing in a mass spectrometer ion source, there would be little point in trying to examine a solution of a sample substance dissolved in one of the common solvents used in chemistry (water, ethanol, chloroform, etc.). Such solvents would evaporate extremely quickly, probably as a burst, on introduction into the ion source. Instead, it is necessary to use a high boiling liquid as solvent (matrix). A low temperature probe has been described, which does utilize low boiling solvents.

In addition to low volatility, the chosen liquid should be a good all-round solvent. Since no one liquid is likely to have the required solvency characteristics, several are in use (Table 1). If a mass spectrum cannot be obtained in one solvent, it is useful to try one or more others before deciding that an FAB spectrum cannot be obtained.

Finally, on bombardment, the solvent itself forms ions which appear as background in a mass spectrum. Very often, protonated clusters of solvent ions can be observed (Figure 5).

The Mass Spectrum

FAB or LSIMS leads to ions being formed from, (a) the sample substance, (b) the matrix or solvent (including clusters) and, (c) general radiation induced fragmentation. A typical example of a substance (M ; molecular mass, 1000) dissolved in glycerol is shown in Figure 6. Protonated molecular ions at m/z 1001 can be observed, together with cluster ions from the solvent (m/z 92, 185, 277...) and a background of randomly fragmented pieces of ionized solvent and substrate in mainly small abundance. This background is sometimes referred to as 'grass'. It does not prove to be a problem usually because the abundances of ions making up the 'grass' are fairly uniform.

Table 1 Some Commonly Used Solvents for FAB or LSIMS

Solvent	Protonated Molecular (m/z) Ions
Glycerol	93
Thioglycerol	109
3-NOBA ¹	154
NOP ²	252
Triethanolamine	150
Diethanolamine	106
Polyethylene glycol (mixtures)	---- ³

1. 3-Nitrobenzyl alcohol
 2. n-Octyl-3-nitrophenyl ether
 3. Wide mass range depending on glycol used.

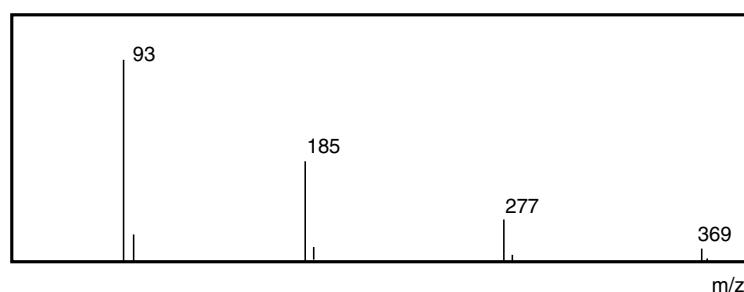


Figure 5 A typical FAB mass spectrum of glycerol alone, showing a protonated molecular ion at m/z 93, accompanied by decreasing numbers of protonated cluster ions (m/z , $l + nx92$; $n=2, 3, 4\dots$).

There can be a problem when the sample under investigation itself gives few molecular ions so that these become impossible or difficult to distinguish against the background. At high molecular mass of the sample the momentum from the bombarding atoms becomes less effective to eject molecular ions which therefore are not abundant and are not easy to discern against the background.

In general, FAB and LSIMS will give excellent molecular mass information in the range (approximately) of m/z 100-2000. Above this value, the abundance of molecular ions tends to be less and less until, in the region of m/z 4000-5000, they become either non-existent or very difficult to discern against the background. Because background ions above about m/z 200-300 tend to have similar and small abundances, FAB or LSIMS mass spectra are often recorded above, say, m/z 200; this practice also eliminates most of the solvent cluster ion peaks (Figure 6b). Alternatively, computer-aided background subtraction can be used to enhance the visibility of molecular ion peaks.

Conclusion

By using a beam of fast atoms or ions incident onto a non-volatile liquid containing a sample substance, good molecular or quasimolecular positive and/or negative ion peaks can be observed up to about 4-5000 Daltons. Ionization is mild and, since it is normally carried out at 25 - 35 °C, it can be used for thermally labile substances such as peptides and sugars.

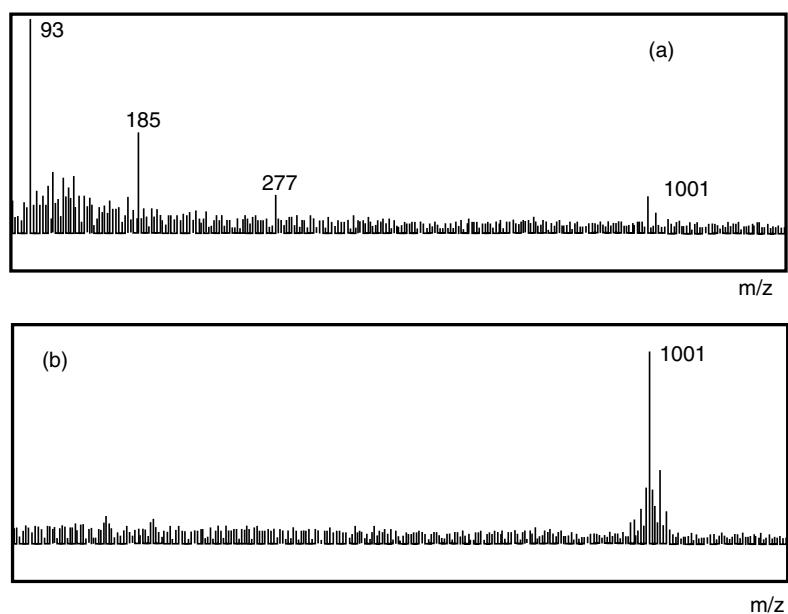


Figure 6 (a) A typical FAB mass spectrum in glycerol with protonated sample molecular ions at m/z 1001, protonated glycerol at m/z 93, protonated glycerol clusters at m/z 185, 277..., and general background ions. The spectrum (b) illustrates the different appearances by recording and expanding from above m/z 300 to m/z 1100. Note the fairly uniform appearances of the background peaks and the absence of solvent cluster ion peaks.

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Quick Guide

- A large electric potential applied to a needle provides a very intense field at its tip where the radius of curvature is small.
- Similarly, a sharp edge (razor blade) or a very sharp curve can also provide an intense electric field.
- For any given electric potential, as the radius of curvature of a tip or edge becomes smaller, the electric field becomes stronger and stronger.
- By growing thin ‘whiskers’ along a sharp edge or thin wire, the ends of the whiskers become regions of very small radius of curvature and, consequently, provide very intense electric fields.
- A molecule (M) lying on one of these tips, experiences the effects of the intense field such that its own electric fields are distorted and the normal barrier to movement of electrons away from or onto the molecule becomes smaller.
- The distortion caused by the field allows an electron to pass from the molecule to the tip if the applied potential is positive or from the tip to the molecule if the potential is negative; this is *field ionization* (FI). The electron transfer occurs through *quantum tunnelling*. Little or no vibrational excitation occurs and the ionization is described as mild or ‘soft’.
- If the applied potential is positive, a positive ion (M^{*+}) is produced and, if negative, a negative ion (M^{*-}) is formed. Since there is no vibrational excitation, no fragment ions are produced.
- A positive ion formed on such a tip held at a high positive potential is repelled and flies off the tip almost immediately after formation and into the mass spectrometer where its m/z value is measured. Similarly, negative ions can be mass measured.
- The above process of field ionization presupposes that the substance under investigation has been volatilized by heat so that some molecules of vapour settle onto the tips held at high potential. In such circumstances, thermally labile substances still cannot be examined even though the ionization process itself is mild.
- To get round this difficulty, a solution of the substance under investigation can be placed on the wire and the solvent allowed to evaporate.

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- Again, when an electric potential is applied, positive or negative ions are produced but no heating is necessary to volatilize the substance. This is *field desorption* (FD) ionization. Both FI and FD provide good molecular mass information but few, if any, fragment ions and allow thermally labile substances such as peptides, nucleosides, and glycerides to be examined, as well as inorganic salts.

Summary

In field ionization (or field desorption), application of a large electric potential to a surface of high curvature allows a very intense electric field to be generated. Such positive or negative fields lead to electrons being stripped from or added to molecules lying on the surface. The positive or negative molecular ions so produced are mass measured by the mass spectrometer.

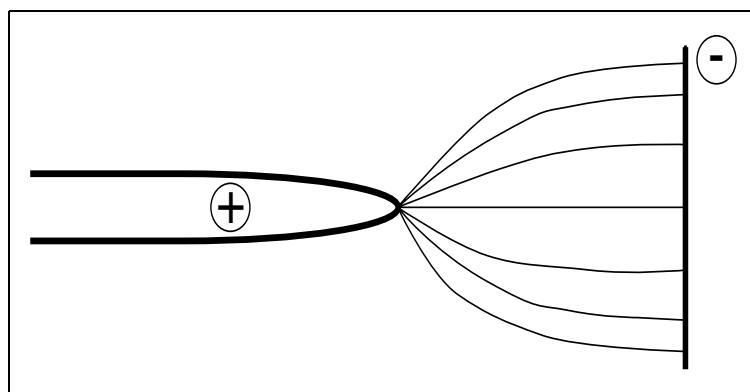


Figure I An electric potential placed across a needle and a flat (plate) electrode. The lines of equipotential in the resulting electric field are focused around the tip of the needle, where the electric field becomes very large.

FIELD IONIZATION AND FIELD DESORPTION

Introduction The main difference between field ionization (FI) and field desorption ionization (FD) lies in the manner in which the sample is examined. For FI, the substance under investigation is heated in a vacuum so as to volatilize it onto an ionization surface. In FD, the substance to be examined is placed directly onto the surface before ionization is implemented. FI is quite satisfactory for volatile, thermally stable compounds but FD is needed for non-volatile and/or thermally labile substances. Therefore, most FI sources are arranged to function also as FD sources and the technique is known as FI/FD mass spectrometry.

Field Ionization If an electric voltage (potential) is placed across an arrangement such as that shown in Figure 1, the lines of equipotential in the resulting electric field crowd in around the needle tip. In this region the field is more intense than, say, near the plate electrode where there are fewer lines of equipotential per unit area. When the needle tip is very fine and the applied potential is large then very intense electric fields can be generated at the surface (point) of the tip. It is in these high field regions that ionization occurs.

Unless extremely high potentials are to be used, the intense electric fields must be formed by making the radius of curvature of the needle tip as small as possible. Field strength (F), is given by equation (1) in which r is the radius of curvature and k is a geometrical factor; for a sphere, k = 1 but for other shapes, k < 1. Thus, if V = 5000 volts and $r = 10^{-6}$ m then, for a sphere, $F = 5 \times 10^9$ V/m; with a larger curvature of, say, 10^{-4} m (0.1mm), a potential of 500,000 volts would have to be applied to generate the same field.

$$F = V/k.r \quad (1)$$

Practically it is easier to produce and apply 5000 volts rather than 500,000.

When a neutral molecule settles onto an electrode bearing a positive charge, the electrons in the molecule are attracted to the electrode surface and the nuclei are repelled (Figure 2), viz., the electric field in the molecule is distorted.

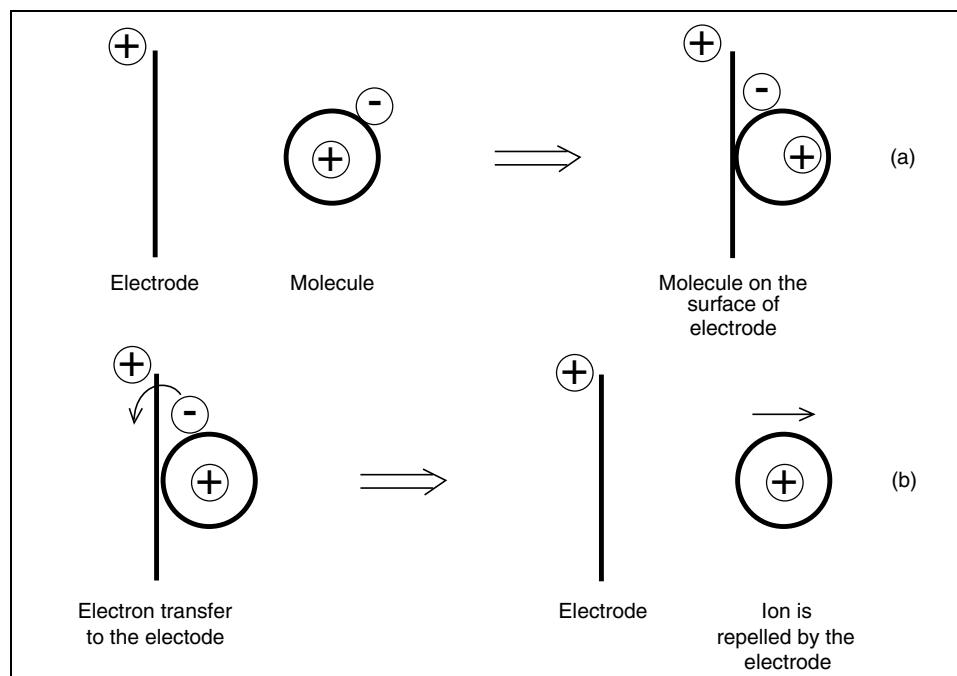


Figure 2 In (a), a molecule alights onto a positive electrode surface, its electrons being attracted to the surface and its nuclei repelled. In (b) an electron has tunnelled through a barrier onto the electrode, leaving a positive ion which is repelled by and shoots away from the positive electrode.

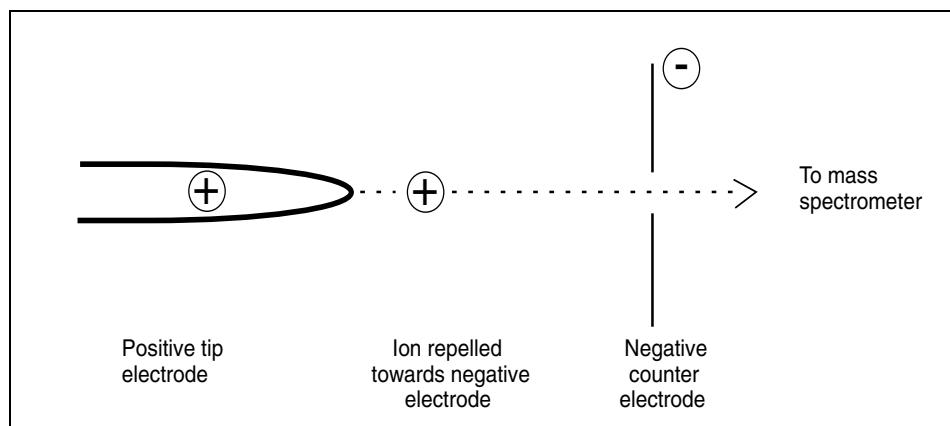


Figure 3 A positive ion formed at a positive electrode tip is repelled and travels towards the negative counter electrode which has a slit in it so that the ion can pass on into the mass spectrometer.

If the electric field is sufficiently intense, this distortion in the molecular field reduces the energy barrier against an electron leaving the molecule (ionization). A process known as quantum tunnelling occurs by which one of the molecular electrons finds itself on the electrode side of the barrier and is promptly neutralized by the positive charges in the electrode. The molecule (M) has then been turned into a positive ion (M^{+}). Because positive charges repel each other, the newly formed positive ion on a positive electrode is repelled by the electrode and flies off into the vacuum of the ion source towards the negative counter electrode (Figure 3). A slit in the counter electrode allows the ion to pass on and into the analyser (mass measurement) part of a mass spectrometer.

The electrical reverse of the above arrangement produces negative ions. Thus, a negative needle tip places an electron on the molecule (M) to give a negative ion (M^{-}) which is repelled towards a positive counter electrode.

Design of the Needle Tip Electrode

If there were only one such tip electrode, the yield of ions would be very limited (small surface area and small numbers of ions formed per unit time). To increase ion yield, it is better to use a lot of tips or microneedles. This might be achieved by using a sharp edge like a razor blade. Indeed, this was one of the first types of ionization sources to be used because even the sharpest razor blade, on a molecular scale, is very rough and has a lot of small tips on its surface (Figure 4). However, such an edge does not provide an efficient ion source. Eventually, methods were found for growing microneedles or 'whiskers' on the surface of a thin wire.

For example, by maintaining the wire at a high temperature in the vapour of benzonitrile, decomposition of the nitrile on the hot wire produces fine, electrically conducting growths or whiskers (microneedles) having tips of very small radius of curvature (Figure 5). Application of a high electric potential to such a wire produces many ionization points and a high yield of ions.

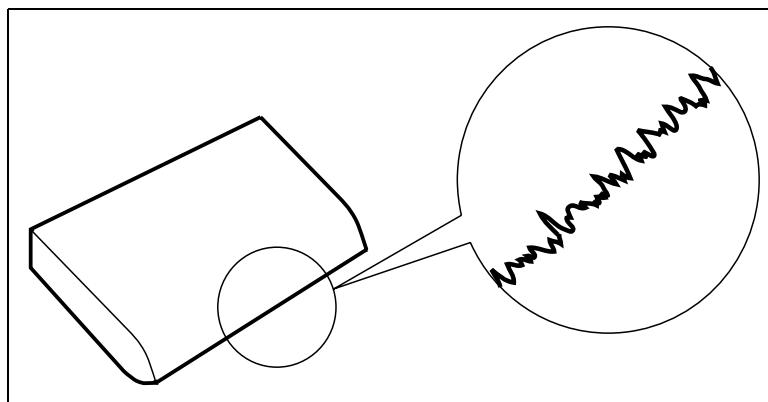


Figure 4 Magnification of (a) a sharp edge showing the many 'tips' and 'valleys' on a molecular scale (b).

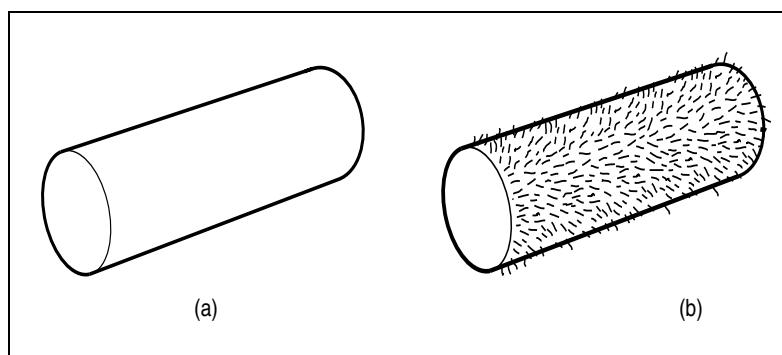


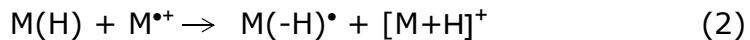
Figure 5 A narrow wire (a) heated in the vapour of an organic compound such as benzonitrile causes decomposition of the nitrile and the formation of whiskery growths on the surface of the wire (b). The sizes of the growths are exaggerated for purposes of illustration and are, in fact, very small in relation to the diameter of the wire.

These thin wires are supported on a special carrier which can be inserted into the ion source of the mass spectrometer after first growing the whiskers in a separate piece of apparatus. Although the wires are very fragile, they last for some time and are easily renewed. They are often referred to as emitter electrodes (ion emitters).

Practical Considerations of Field Ionization/Field Desorption

Although there has been some controversy concerning the processes involved in field ionization mass spectrometry, the general principles appear to be understood. Firstly, the ionization process itself produces little excess of vibrational and rotational energy in the ions and, consequently, fragmentation is limited or non-existent. This ionization process is one of the mild or 'soft' methods available for producing excellent molecular mass information. The initially formed ions are either simple radical-cations ($M^{\bullet+}$) or radical-anions ($M^{\bullet-}$).

However, in both FI and FD, there are other neutral molecules on or close to the surface of the emitter and, in this region, ion/molecule reactions between an initial ion ($M^{\bullet+}$) and a neutral ($M(H)$) can produce protonated molecular ions ($[M+H]^+$; equation 2).



For simple FI, the substance to be mass measured is volatilized by heating it close to the emitter so that its vapour can condense onto the surface of the electrode. In this form, an FI source can be used with gas chromatography, the GC effluent being passed over the emitter. However, for non-volatile and/or thermally labile substances, a different approach must be used.

Now, a solution of the substance to be examined is applied to the emitter electrode by means of a microsyringe, outside the ion source. After evaporation of the solvent, the emitter is put into the ion source and the ionizing voltage is applied. By this means, thermally labile substances such as peptides, sugars, nucleosides and so on can be examined easily and provide excellent molecular mass information.

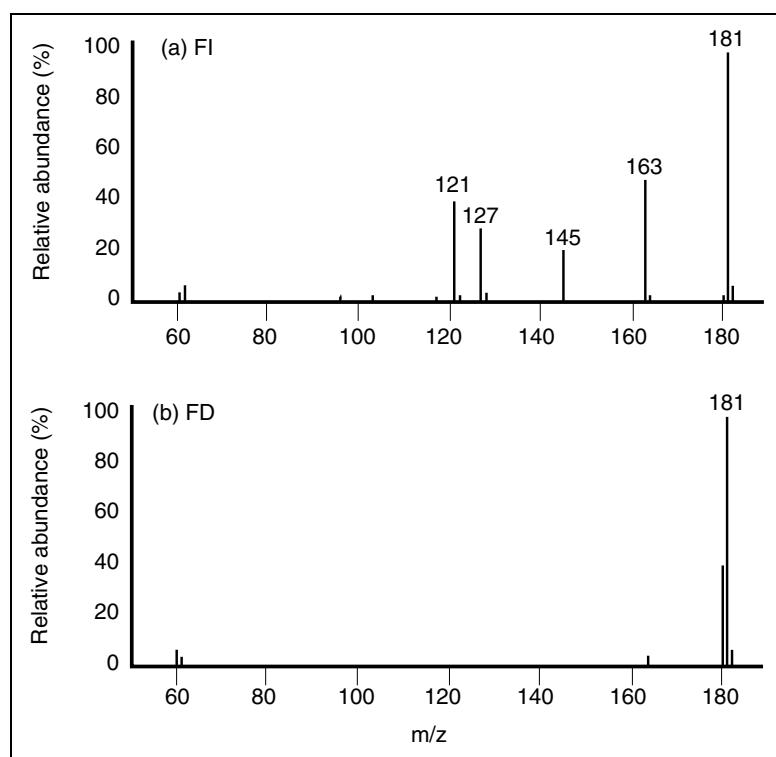


Figure 6 A comparison of the (a) FI and (b) FD spectra of D-glucose. Note the greater fragmentation in FI (heat applied for volatilization) and the appearance of M^{*+} ions at m/z 180 in the FD spectrum as well as the $[M+H]^+$ ions at m/z 181 in both spectra.

Although still FI, this last ionization is referred to specifically as field desorption. A comparison of FI and FD spectra of D-glucose is shown in Figure 6.

By intentionally adding inorganic salts to the solution used for FD, cationated molecular ions can be produced in abundance. Equation (3) illustrates how addition of NaCl can give rise to $[M+Na]^+$ ions.



Sometimes, in FD, the emitter electrode is heated gently either directly by an electrode current or indirectly by a radiant heat source to aid desorption of ions from its surface.

Types of Compounds Examined by FI/FD

Newer developments in ionization methods have tended to overshadow FI, particularly in view of the relatively fragile nature of the emitters and the need for separate apparatus in which to form the microneedles. In contrast, FD still offers advantages, being able to ionize a wide range of mass spectrometrically difficult substances (peptides, sugars, polymers, inorganic and organic salts, organometallics).

Because there is little fragmentation on FD, it is necessary to activate the molecular or quasimolecular ions if molecular structural information is needed. This can be done by any of the methods used in tandem MS as, for example, collisional activation (see Back-to-Basics guides on tandem MS and collisional activation).

Conclusion

FI and FD are mild or ‘soft’ methods of ionization which produce abundant molecular or quasimolecular positive or negative ions from a very wide range of substances. In the FD mode, it is particularly useful for high molecular mass and/or thermally labile substances such as polymers, peptides and carbohydrates.

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Back to Basics Section A: Ionization Processes

CHAPTER A6

CORONAS PLASMAS AND ARCS

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Quick Guide

- Extra energy can be added to atoms, molecules and ions; they become energetically excited.
- For atoms, molecules and ions, the extra energy can make them move faster (this is an increase in kinetic or translational energy).
- For molecules and ions having more than one atom, the extra energy can make the component bonds rotate and vibrate faster (this is rovibrational energy). Isolated atoms, having no bonds cannot be excited in this way.
- For atoms, molecules and ions, the extra energy may be sufficient to cause one or more electrons to move from one orbital to another (electronic excitation).
- As excited atoms, molecules or ions come to equilibrium with their surroundings at normal temperatures and pressures, the extra energy is dissipated to the surroundings. This leads to the particles slowing down as translational energy is lost, rotating and vibrating more slowly as rovibrational energy is lost and to them emitting light or X-rays as electronic energy is lost.
- The loss of energy returns the particles to their original (ground state), viz., their energy state at normal temperatures and pressures.
- For electronically excited species, the emitted light may be used for spectroscopic purposes, as in fluorescence analysis.
- If an electric potential is applied between two electrodes in a gas, electrons are released from the cathode (negative electrode). The electrons are accelerated by the electric field and collide with atoms or molecules of gas.
- These collisions may be sufficiently energetic that the gas molecules become electronically excited and, as the excited electrons return to their ground state, they emit light. Thus, passage of electrons (an electric current) through a gas under the right conditions leads to the emission of light from the gas.
- The colour of the emitted light depends on what type of gas is present. For example, sodium atoms may glow with a yellow light (as in the familiar yellow street lights) and neon may glow with a dark red light (as in the familiar neon lights).

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- The appearance of light during passage of a current through a gas (called a discharge) is a manifestation of electronic excitation but the conditions for excitation are dependent on the pressure of the gas and the voltage applied to cause the discharge. At low voltages there is no discharge and, usually, some 200–800 volts are needed to start the discharge process.
- As the voltage is increased, intermittent discharges occur because the discharge loses energy to its surroundings. Eventually, the discharge becomes self-sustaining and, by maintaining a constant current flow, the discharge continues and light is emitted until the power is switched off.
- The exact conditions of gas pressure, current flow and applied voltage under which the discharge occurs determines whether or not it is of the corona, plasma or arc type. The colour of the emitted light may also change, depending not only on the type of gas used, but also on whether it is a corona, plasma or arc discharge.
- All of the types of discharge involve the formation of ions as part of the process. For various reasons, ions are mostly positive ones. The ions may be examined by mass spectrometry. If small amounts of a sample substance are introduced into a corona or plasma or arc, ions are formed by the electrons present in the discharge or by collision with ions of the discharge gas.
- Thus, either the emitted light or the ions formed may be used to examine samples. For example, the mass spectrometric ionisation technique of Atmospheric Pressure Chemical Ionisation (APCI) utilises a corona discharge to enhance the number of ions formed. Carbon arc discharges have been used to generate ions of otherwise analytically intractable inorganic substances, the ions being examined by mass spectrometry.
- Since a discharge is characterised by having a substantial population of charged species (electrons and ions), it responds to an applied electromagnetic field. The applied field moves electrons in one direction and positive ions in the opposite direction, in accordance with Maxwell's Laws.
- If the applied electromagnetic field is an alternating one, then the electrons and ions are pushed (or pulled) backwards and forwards as the sign of the field changes. At high frequencies of applied fields, this motion causes multiple collisions between ions and neutral species and between electrons and ions and neutral species.

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- The multiple energetic collisions cause molecules to break apart, eventually to form only atoms, both charged and neutral. Insertion of sample molecules into a plasma discharge, which has an applied high frequency electric field, causes the molecules to be rapidly broken down into electronically excited ions for all of the original component atoms.
- This is the basic process in an inductively coupled plasma discharge (ICP). The excited ions may be examined by observing the emitted light or by mass spectrometry. Since the molecules have been broken down into their constituent atoms (as ions) including isotopes, these may be identified and quantified by mass spectrometry, as happens with isotope ratio measurements.

Summary Depending on gas type and pressure, by application of an electric potential across two electrodes in the gas, an electric discharge can be maintained in the gas, producing ions and emitted light. Whether the discharge is corona, plasma or arc depends on the voltage and current flowing through it. The discharge contains electrons and ions and the latter may be examined mass spectrometrically.

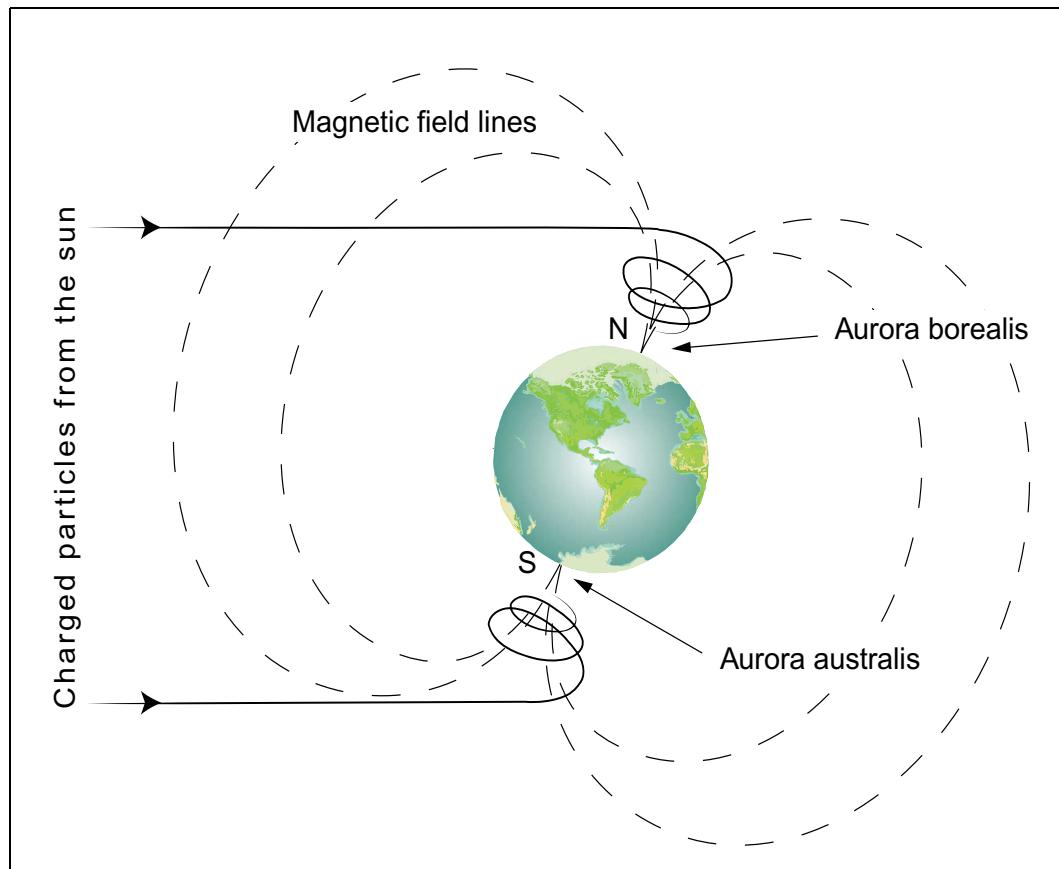


Figure I. Schematic representation of the movement of positively charged particles (mostly protons) from the sun entering the Earth's magnetic and electric fields. Under the influence of the magnetic component, the particles spiral down towards the north and south poles of the negatively charge Earth, clockwise at one pole and anticlockwise at the other. When the incoming charged particles collide with molecules of oxygen, nitrogen, argon, water and other gas molecules in the upper atmosphere, excitation occurs and this leads to emission of light, known as the Aurora Borealis in the northern hemisphere and the Aurora Australis in the southern.

CORONAS PLASMAS AND ARCS

Background

Charged particles, usually protons, from the sun encounter the earth's magnetic field and spiral down towards the negatively charged earth and meet the atmosphere above the magnetic north and south poles (Figure 1). The charged particles from the sun are moving at high speed and begin to collide with molecules of oxygen, nitrogen and other gases in the upper atmosphere. These high energy collisions cause electrons in the gas molecules to be excited into higher energy orbitals to form excited atoms or ions (Figure 2). Additionally, nitrogen and oxygen ions are formed when an electron is ejected from a molecule altogether by the energy of collision. During this process, the remaining electrons in the ions may also be excited. Other gases are present in the atmosphere such as carbon dioxide, argon and so on and these also form excited molecules and ions. When the electrons in the excited atoms or ions return to their original (ground) state, light is emitted which may be green, red or other colours depending on which molecules have been excited (Figure 3). Such events lead to the appearance of the very pretty, mysterious displays of lights in the sky in the polar regions of the northern and southern hemispheres, viz., the *aurora borealis* and *aurora australis* (sometimes the light can be observed in areas somewhat more distant from the poles and, in the north, they are called 'northern lights'). These natural phenomena are manifestations of electric discharge physics, namely the passage of charged particles through a gas. Excitation of atoms or molecules in an electric field by electrons and recombination of ions and electrons causes the formation of excited species, which emit light. Other examples of the discharge may be seen in lightning flashes, the common yellow sodium street lights, fluorescent lighting generally and arc welding.

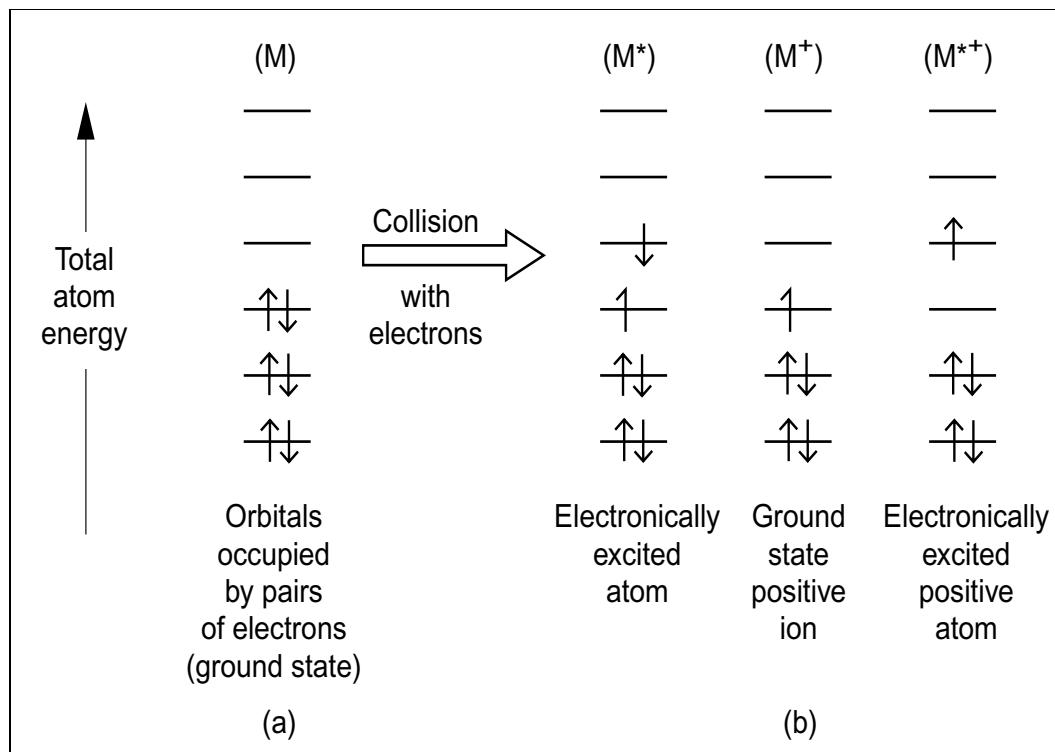


Figure 2. (a) The diagram shows occupied and unoccupied orbital energy levels in an atom. Some of these are occupied by pairs of electrons (the electron spins in the bonding levels are indicated by the arrows) and some are empty in the normal ground state of the atom (M). After collision with a high energy (fast moving) electron, one or more electrons are promoted to higher unoccupied orbitals. In (b), one electron is shown as having been promoted to the next higher orbital energy to give an electronically excited atom (M^*). Alternatively, the promoted electron may be ejected from the atom altogether, leaving a ground state positive ion (M^+). Finally, an ion may be formed by loss of an electron but, at the same time, another electron may be promoted to a higher orbital to give an electronically excited ion (M^{*+}).

In the laboratory, it has been found that similar effects can be produced if a voltage is applied between two electrodes immersed in a gas. The nature of the laboratory or instrumental discharge depends critically on the type of gas used and on its pressure and the magnitude of the applied voltage. The actual electrical and gas pressure conditions determine whether or not the discharge is called a corona, a plasma or an arc.

Although the discharges attract interest because of the emitted light, it should be recalled that major components of the discharges are ions and electrons. The electrons may be utilised in mass spectrometry to enhance ionisation of sample molecules and the ions themselves may be used to gain information about the sample (m/z values and abundances of ions). It is this last use of discharges, which is discussed in this present Back-to-Basics article. The effects of electrons in these discharges may be greatly enhanced by the application of an external high frequency electromagnetic field, which leads to the plasma discharge reaching very high temperatures as in plasma 'torches'.

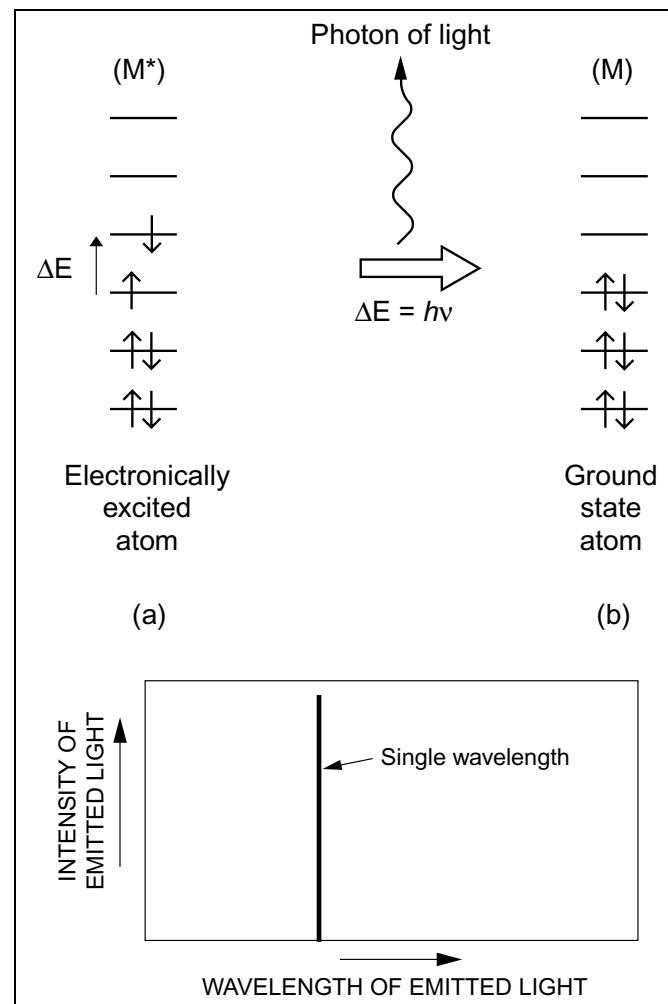


Figure 3. (a) The orbital levels of an electronically excited atom (or molecule) show one electron in a higher orbital than it would normally occupy. (b) The atom may return to its normal ground state by emitting a photon of light of frequency (ν), for which $\Delta E = h\nu$ is the energy change in the atom as the photon is emitted and the electron drops to the more stable state. Since ν is determined by ΔE , the wavelength (λ) of the light is found by dividing the speed of light (c) by the frequency ν , viz., $\lambda = c/\nu$. If λ falls in the visible region of the electromagnetic spectrum (approximately 400–700 nm), the emitted radiation appears as coloured light of a single wavelength. The colours of the emitted light depend on the nature of the gas atoms or molecules that are excited.

**Electric discharges
in a gas**

In this discussion, only inert gases such as argon or neon are used as examples because they are monatomic and this simplifies description of the excitation. The introduction of larger molecules into a discharge is discussed later in other Back-to-Basics guides concerning examination of samples by mass spectrometry.

If a gas such as argon is held in a glass envelope, which has two electrodes set into it (Figure 4), application of an electric potential across the electrodes leads to changes in the gas, which result from the passage of electrons from the cathode (negative electrode) to the anode (positive electrode). This passage of electrons is highly dependent on the type of gas present, the pressure of the gas and the voltage applied. The most obvious demonstration of the flow of electrons in the gas arises from the emitted light (Figure 4). As described above, this light comes partly from the formation of excited atoms and ions in the gas (Figure 3) and partly from their recombination with electrons (Figure 5). Unlike atom excitation, which mostly gives rise to light being emitted at one or two fixed wavelengths, the recombination process leads to a 'band' spectrum of emitted light, in which there is a wide spread of very closely spaced wavelengths (Figure 5).

A fuller description of the discharge process follows as a typical example, in which a voltage is applied to the electrodes and is then gradually increased.

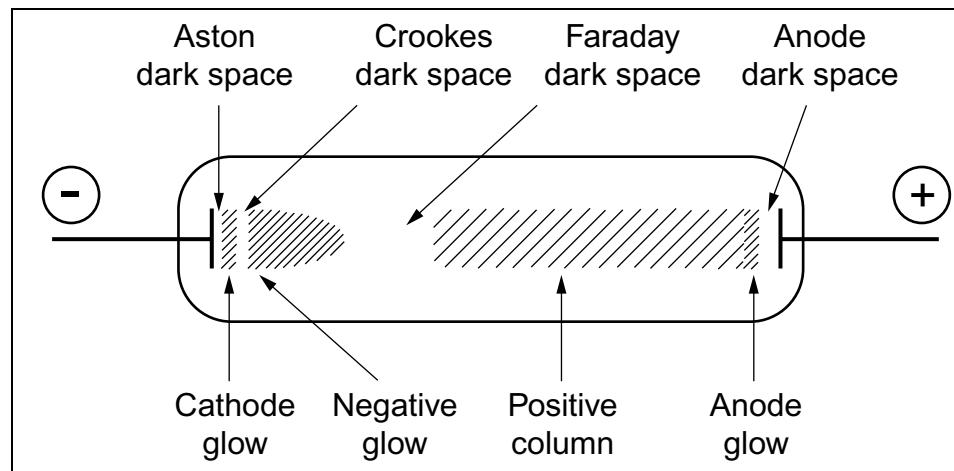


Figure 4. A typical representation of light emitting regions and dark spaces in a gas discharge between two electrodes, one electrically negative (the cathode), the other positive (the anode). The glowing regions are known as the cathode glow, the negative glow, the positive column and the anode glow. The regions emitting no light are named after earlier pioneers of gas discharges, viz., the Aston, Crookes and Faraday dark spaces. Actually the dark spaces do emit some light but appear dark in contrast to the much brighter glow regions. The discharge takes up a form controlled by the shapes of the electrodes and, in this case, it is a cylindrical column stretching from one disc-like electrode to another.

Table I. Typical colours of light emitted in different regions of the glow discharge.

Gas used	Cathode glow	Negative glow	Positive glow
Nitrogen	pink	blue	red
Oxygen	red	yellow/white	pale yellow
Air	pink	dark blue	pink
Neon	yellow	orange	red
Argon	pink	dark blue	dark red
Sodium	pink	white	yellow
Mercury	green	green	green

The colours shown above are only approximate. Sometimes mixtures of colours are seen as gas pressure or applied voltages change or if impurities are present

Light and dark regions in the discharge

(i) Aston dark space and the cathode glow

The light regions result from electron collisions with neutral atoms in the gas and from recombination of electrons and positive ions to give atoms.

The energy of an electron is controlled by its velocity, which is proportional to the applied voltage. Electrons emitted from the cathode are accelerated by the electric field. As they cross the Aston dark space the electrons gather speed and collide with atoms of gas. Across the Aston dark stage the electrons have insufficient energy to excite gas atoms, any colliding electron and atom pair simply 'bouncing' away from each other without any overall transfer of energy other than kinetic. This sort of collision is said to be elastic if no energy is interchanged. Because there is insufficient energy transfer to cause electronic excitation in the neutral atom, this region of the discharge is dark. As the speed of an electron reaches a certain threshold level and collision occurs with an atom, the electric field of the electron and the electric field of the electrons in the atom interact with each other. The transferred energy causes one or more electrons in the atom to be promoted to a higher energy level (the atom is energetically excited). The excited state is unstable and quickly loses energy to return to the ground state by emitting a photon ($h\nu$). If ν lies in the visible region of the electromagnetic spectrum then the phenomenon is manifested by the production of light. This is basis of the cathode glow shown in Figure 4. The colour of the light emitted depends on ν , which in turn depends on the nature of the gas atoms that are excited (Figures 3,5). Light is also emitted as a broad band spectrum following electron/ion recombinations (Figure 5). The typical excitation colours shown in Table I are caused by a superposition of 'line' emissions from excited atoms and broad band emission from electron/ion recombination processes.

Because the colliding electron loses energy (slows down) after an inelastic collision, it no longer has sufficient energy to excite any more gas atoms and so the cathode glow appears as a fairly well defined band (Figure 4), the front of which indicates the place where the colliding electrons have sufficient energy to cause emission from the gas atoms and the back (furthest from the cathode) of which marks the region where the electrons have lost energy in collisions and can no longer excite the gas. This last point is also the beginning of the Crookes' dark space.

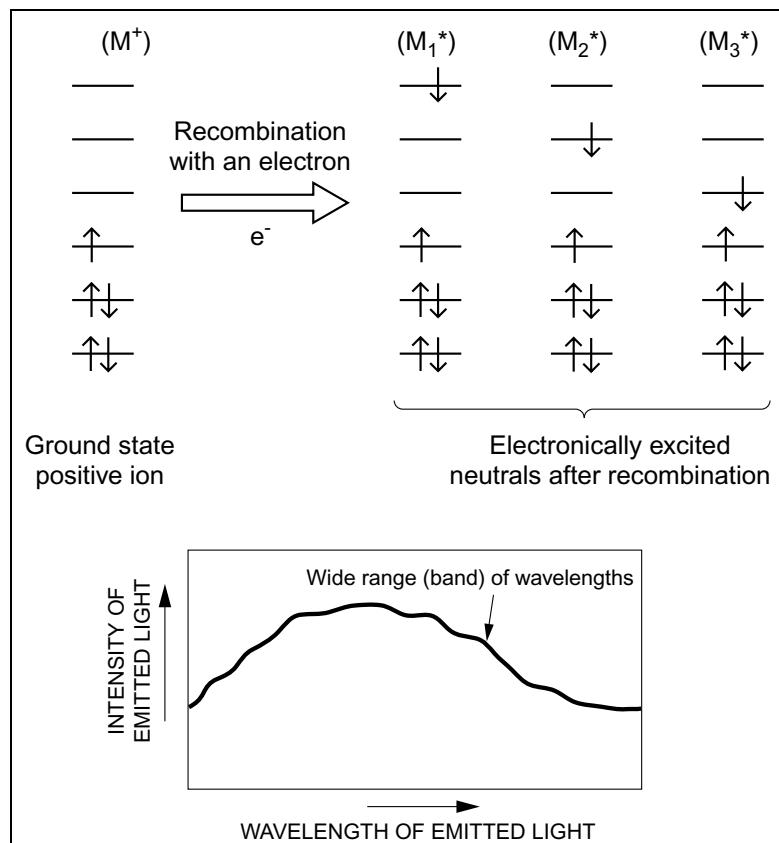


Figure 5. When an electron recombines with a positive ion, the incoming electron will attach at any of the vacant atomic orbitals, as illustrated by the three typical states (M_1^* , M_2^* , M_3^*). Other more excited states may be formed as the electron distribution in the newly formed neutral is disturbed ('shaken up') by the added energy of the incoming electron. However, most of these states are unstable with respect to the ground state atom and the disturbed electrons drop down to more stable orbitals until the ground state is reached. This increase in stability can only be achieved if energy is lost from the system. Thus, each time an electron drops to a lower orbital, a quantum of light is emitted, which has a wavelength that depends on the spacing between orbitals. Since many of these excited states may be formed on recombination and decay of the initial excited states, the spectrum of emitted light covers a wide range of wavelengths, viz., it is broad band emission. The light emitted from a gas discharge is a superposition of the line spectra arising from directly excited neutral atoms (Figure 3) and broad band spectra from electron/ion recombination. Some typical colours of emitted light are given in Table I.

(ii) Crookes' dark space and the negative glow

After leaving the cathode glow, some of the electrons originally emitted from the cathode have slowed down but others have suffered few collisions and are travelling considerably faster (have more energy). In the Crookes' dark space there is an assemblage of electrons of various energies being accelerated by the external electric field between the electrodes. In the dark space, all of the electrons are accelerated and two major processes (a, b) occur.

In process (a), slow electrons are accelerated until they have sufficient energy to again excite gas atoms; this is the start of the negative glow, just like the process in the cathode glow (Figure 4).

In process (b), fast electrons are accelerated to even higher speeds (higher energy), eventually being able to remove an electron from an atom entirely, *viz.*, the atom is ionised (Figure 2b). In this sort of inelastic collision between an atom and an electron, two electrons leave the collision site (Figure 6). The positive ion that is produced will be considered later. However, the two electrons leaving the collision site are accelerated and eventually are able to either cause an atom to emit light (process a) or to cause ionisation (process b). Thus the negative glow is a region, in which a cascade of electrons is produced by ionising collisions, which are in addition to the original flux of electrons coming from the cathode. This cascade of electrons leads to many inelastic collisions with gas atoms and therefore to the emission of many photons of light. Thus, the negative glow is much brighter than the cathode glow and spreads over a larger region of space. Also, because of the initial large spread of energies as electrons leave the Crookes' dark space and begin causing the negative glow, the back end of the glow is not 'sharp' as with the cathode glow but gradually fades out into the following Faraday dark space. The spread of energies of electrons going into the negative glow means that the light emitted results from a range of excitation and recombination possibilities and is usually a different colour from that seen from the cathode glow. Although this region of the negative glow is known as a corona, this term is now usually applied when inhomogeneous electric fields are used in gases at or near atmospheric pressure, in which the field is sufficient to maintain a discharge but is unable to produce much of a glow, or even none at all.

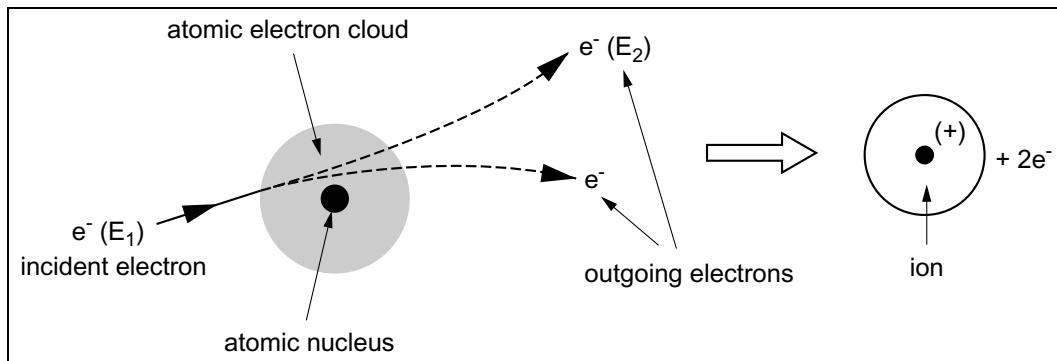


Figure 6. After acceleration through a voltage (V), an electron has a new velocity (u) and energy ($e \times V$), which is equal to the kinetic energy gained by the electron ($mu^2/2$). This is the energy (E_1) of the incoming electron shown in the diagram ($E_1 = eV = mu_1^2/2$). After 'collision' with the atom, two electrons leave the reaction site, the original electron having lost some of its energy (now, $E_2 = mu_2^2/2$). The other newly ejected electron has energy equal to the difference between the ionisation energy (I) of the atom and the energy lost by the incident electron ($E_2 = E_1 - I$). Since energy has been given up, $E_2 < E_1$ and $u_2 < u_1$, viz., the exiting electron leaves the collision slower than it moved as an incoming electron. It should be recalled that, in the collision process between the incident electron and the atom, the electron is so small and the 'electronic' space surrounding the nucleus of the atom is so relatively large that there is no collision as such. Rather, the incident electron with its associated wave-like electric field, perturbs the electrons in the atom as it passes through the relatively vast open spaces of the atom's electron cloud. If the perturbation is sufficiently large, one of the atom's electrons will be ejected completely along with the departing (originally incident) electron.

(iii) Faraday dark space and the positive column

After leaving the negative glow, electrons have insufficient energy for either exciting or ionising effects but they begin to be accelerated again. This is the Faraday dark space. It should be noted that, in the region from the cathode to the start of the negative glow, the electric field resulting from the applied voltage on the electrodes is high and changes rapidly for reasons discussed below under the heading of space charge. However, from the end of the negative glow to the anode, the electric field gradient is small and almost constant; electrons leaving the negative glow are only slowly accelerated as the electrons move towards the anode. In this region, inelastic collisions are less frequent but ionising collisions do still occur and also there are some collisions leading to emission of light. Thus, the positive column emits light less strongly than does the negative glow and often the light is a different colour too (Table 1). The positive column is a region, in which atoms, electrons and ions are all present together in similar numbers and it is referred to as a plasma. Again, as with the corona discharge, in mass spectrometry, plasmas are usually operated in gases at or near atmospheric pressure.

(iv) Anode dark space and anode glow

Positive ions formed near the positive electrode (anode) are repelled by it and move into the positive column. Electrons, which reach proximity to the anode, are accelerated somewhat because the electric field gradient increases slightly. The more energetic electrons cause more emission of light near the anode than from the main body of the positive column and so this end region appears brighter than the main body of the positive column. This is the anode glow. Sometimes this glow is not very marked. Also in this region, inelastic collisions with atoms lead to the formation of more positive ions, which are repelled from the anode and into the positive column. After these inelastic collisions with gas atoms, the electrons have lost energy but continue on to the anode and are discharged. The last region of the discharge contains electrons with too little energy to cause excitation and no light is emitted; this is the anode dark space.

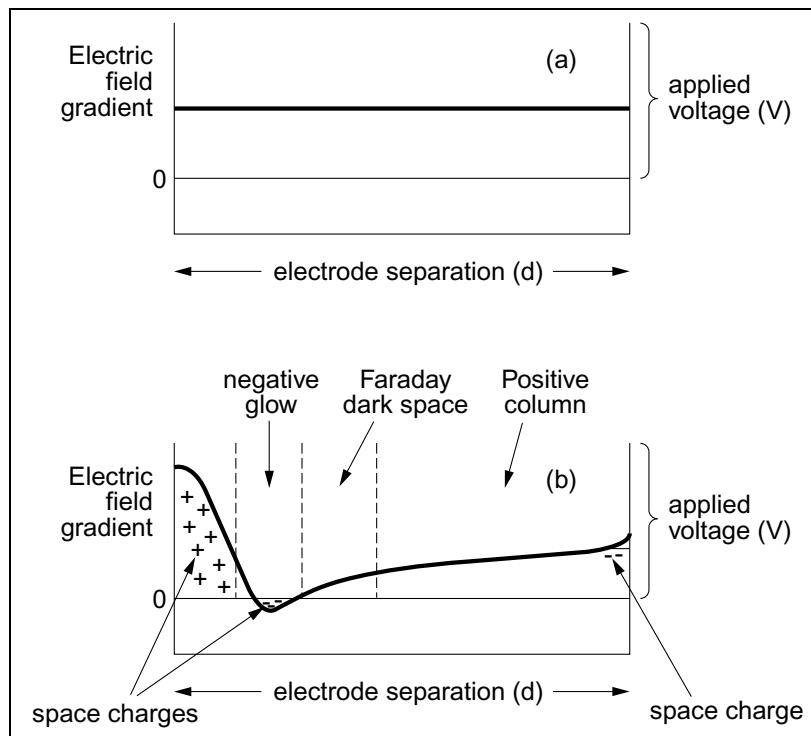


Figure 7. In (a), before any discharge occurs, the voltage (V) has been applied across two electrodes, distance (d) apart. The electric field has a constant gradient ($= V/d$). In (b), the discharge has been set up. Because of space charge effects, in which there is a preponderance of positive ions or negative electrons, the electric field is no longer uniform. Between the cathode and the start of the negative glow, there is a large fall in potential through the positive space charge region, leading to a large field gradient. This is a region, in which electrons are strongly accelerated. Just in front of the negative glow, an excess of electrons leads to a small negative space charge as the gas density of electrons begins to rise to be greater than the gas density of positive ions. Beyond the negative glow, there is a much smaller change in the field gradient because there are almost equal numbers of ions and electrons. Only near the anode is there another small (electron) space charge effect, which accelerates ions away from the anode.

Electric field gradients across the glow discharge

In Figure 4, the two electrodes are marked as cathode and anode, arising from the application of an external voltage between them. Before any discharge occurs, the electric field gradient between the electrodes is uniform and is simply the applied voltage divided by the their separation distance. This is shown in Figure 7. When the discharge has been set up, there is a movement of electrons from cathode to anode and a corresponding movement of positive ions from the anode to cathode. These transfers of electrons and ions to each electrode must balance to maintain electrical neutrality in the circuit. Thus, the number of positive ions discharging at the cathode must equal the number of electrons discharging at the anode. This occurs but the actual drift velocities of electrons and ions towards the respective electrodes are not equal. As the electrons move from cathode to anode, they undergo elastic and inelastic collisions with gas atoms. The paths of the electrons are not along straight lines between the electrodes because of the collisions. In effect, the movement of each electron consists of short steps between collisions, some of which will even cause the electron to move backwards. The overall electric field reverses any such backward recoil. There is a 'random walk' as the electrons gradually make their meandering way across the discharge region. In a perfect vacuum, the velocity u of each electron would be given by the formula, $mu^2 = 2eV$, in which m is the mass of an electron, e is the electronic charge and V is the applied potential. The time t taken to cross the distance d between the electrodes is then simply $t = d/u$. However, because of the meandering path caused by frequent collisions, the average speed (the drift velocity) is much smaller and the time taken to reach the anode is much longer than would be the case in the absence of any neutral gas molecules (in a vacuum). This sort of movement of particles in gases and liquids is common and is normally referred to in terms of a mobility of the particle. The mobilities of positive ions are about 100 times less than the mobilities of electrons. This means that positive ions move more slowly in the anode-to-cathode direction than electrons move in the opposite direction. Since only the same numbers of ions and electrons can be discharged in unit time, the result of the difference in mobilities is that a large excess of positive ions gathers near the cathode and a much smaller excess of electrons gathers near the anode. These clouds of ions and electrons around the electrodes constitute space charges and mean that the electric field gradient is large near the cathode but is much smaller near the anode (Figure 7). The effect of the positive space charge resembles the effect of moving the anode closer to the cathode. Most of the voltage difference applied to the electrodes falls across a narrow region close to the cathode (Figure 7). This is the reason that

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electrons, generated at the cathode, are rapidly accelerated into the cathode and negative glow regions but are only slowly accelerated along the positive column. From the negative glow to near the anode dark space, the numbers of ions and electrons are very similar and, in this region, the electric field gradient is only small, making acceleration of electrons and ions very small. In the region close to the anode, there is a small increase in the electric field gradient that accelerates the electrons towards the anode and ions away from it. The full profile of the electric field is illustrated in Figure 7.

Self-sustaining discharge

Once the glow discharge has begun, a number of processes is set in train to maintain it. Before discharge begins, the cathode emits few electrons unless it is heated (see Back-to-Basics guide on *Thermal Ionisation*) or unless light is shone on it (photoelectric effect). However, once light is being emitted from the discharge glow, the light falling on the cathode induces a photovoltaic emission of more electrons, thereby enhancing the flow of electrons from the electrode.

In addition to this source of extra electrons, there is a ‘bombardment’ of the cathode by the incoming positive ions. As the positive ions plunge into the surface of the electrode their kinetic energies are transferred to the constituents of the electrode metal. This momentum transfer causes the emission of secondary electrons and other species, which again improves the flow of electrons. The glow discharge leads to more electrons being released from the cathode than would be the case otherwise and the total current flow through the discharge increases. The glow becomes selfsustaining as long as an electric potential exists across the electrodes. Thus, the starting voltage needed to set up a glow discharge can be reduced once the discharge is underway. A description of the current/voltage changes in a typical discharge is given below.

Sputtering

Ions impacting onto the cathode during a discharge cause secondary electrons and other charged and neutral species from the electrode material to be ejected. Some of these other particles derived from the cathodic material itself migrate (diffuse) to the walls of the discharge tube and form a deposit there. This effect of transferring material from an electrode to other parts of the discharge system is called sputtering.

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Effect the electrode separation on the discharge

At any one gas pressure, the separation between the electrodes determines the appearance of the discharge. At low pressures of about 1 Torr, the appearance of the discharge is similar to that shown in Figure 4. If the electrodes are moved further apart, a greater voltage becomes necessary to maintain the discharge. The positive column increases in length but there is no effect on the cathode regions because the space charge maintains the high electric field gradient near the electrode. The positive column therefore simply increases in length as the positive ions and electrons within it have further to migrate.

If the electrodes are moved closer together, the positive column begins to shorten as it moves through the Faraday dark space because the ions and electrons within it have a shorter distance through which to diffuse. Near the cathode however, the electric field gradient becomes steeper and electrons from the cathode are accelerated more quickly. Thus, atom excitation through collision with electrons occurs nearer and nearer to the cathode and the cathode glow moves down towards the electrode.

Arcs

As the voltage across the discharge is increased, the glow discharge gets brighter and the current rises as more and more electrons are released through ionisation and through bombardment of the cathode by more ions. The negative glow is then almost on top of the cathode, the separation between it and the cathode itself being much less than a millimetre. The positive column or plasma glow increases as the plasma spreads to occupy almost all of the space between the electrodes. At some point, the cathode glow suddenly becomes a bright spot on the cathode and the voltage falls as the current flowing increases again. This is when an arc is struck. There is a very bright narrow column of hot gas between the electrodes. The reason for the fairly sudden increase in the flow of electrons as the arc is struck probably arises from three sources. One is an increase in the numbers of secondary electrons emitted from the cathode under increased bombardment from the larger numbers of positive ions being produced. Another results from increased thermal emission of ions as the cathode heats up and a third arises from field emission (see Back-to-Basics guide on *Field Ionisation*). As the negative glow approaches ever nearer (10^{-6} m) to the cathode, the electric field gradient between the cathode and the glow becomes very high, reaching 10^8 to 10^9 volts/metre for an applied potential of 100 volts. This electric field condition is in the region of that required for field ionisation.

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A typical temperature in an arc is about 2000 K. An arc can be struck in other ways as in welding or arc lights. For such uses, two electrodes are first touched together (very low electrical resistance) and a relatively small potential is applied. Because the resistance is small, a large current passes between the electrodes and a rapid sequence of events, as described above for the glow discharge, ends with an arc being formed. If the electrodes are then drawn apart, there is an increase in resistance and the current density settles. The high current density in the arc causes rapid heating of gas molecules and the emission of large amounts of light. Arcs are usually struck in gases at atmospheric pressure. The intensity of the emitted light is used for the bright arc lights in theatres but, for a welder, the intensity of light and the wavelengths it covers, including the ultraviolet, would damage his/her eyes, which must be shielded with dark glass. Material sputtered from the electrodes is used in the weld. The arc discharge has been used to volatilise and ionise thermally intractable inorganic materials such as bone or pottery so that a mass spectrum of the constituent elements can be obtained.

Effect of gas pressure on the discharge

The appearance of the glow discharge at about 1 Torr is shown in Figure 4. If the gas pressure is reduced, the space charge is reduced and electrons emerging from the cathode have further to travel to collide with gas atoms. Thus, the cathode glow moves away slightly from the cathode but the negative glow moves strongly towards the Faraday dark space because the cascade of electrons formed by a collisional process has to travel further to meet a sufficient number of gas atoms. At the same time, the positive column shortens, appearing to disappear into the anode region and becoming weaker. If the gas pressure continues to be reduced, there will be too few electron/atom collisions to maintain a cascade of electrons and the discharge stops (goes out) unless the voltage is increased.

If the gas pressure is increased, the opposite effects occur. The negative glow, the cathode glow and the dark spaces move towards the cathode and the positive column gets longer. The lengthening effect of the positive column essentially brings the anode nearer to the cathode. At about 100–200 Torr, the negative glow moves almost up to the surface of the cathode, followed by the Faraday dark space. The positive column not only lengthens but begins to weaken too as it becomes fainter and narrower.

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Effect of gas flow on the discharge

With a discharge tube totally enveloping the discharge gas, there is a faster drift of electrons than ions to the walls of the tube, which become negatively charged. Positive ions and sputtered materials are attracted there and this reduces the flow of current in the discharge. The build-up of a deposit may eventually lead to most electrons and ions moving to the walls of the tube rather than to the electrodes and the discharge stops. This situation occurs in the common 'fluorescent' tubes used for lighting. In scientific apparatus, in which coronas and plasmas are struck, it is more usual to have a continuous flow of gas through the discharge region to help prevent a build-up of deposits.

Effect of electrode shapes on the discharge

Particularly in mass spectrometry, where discharges are used to enhance or produce ions from sample materials, mostly coronas, plasmas and arcs are used. The gas pressure is normally atmospheric and the electrodes are arranged to give non-uniform electric fields. Usually, coronas and plasmas are struck between electrodes that are not of similar shapes and this complicates any description of the discharge because the resulting electric field gradients are not uniform between the electrodes.

In Atmospheric Pressure Chemical Ionisation, a nebulised stream of droplets leaves the sample inlet tube and travels towards the entrance to the mass analyser. During this passage, ions are produced but the yield is rather small. By introducing electrodes across the flow of sample material at atmospheric pressure a discharge can be started, which is essentially of a corona type. In this discharge, electrons and positive ions are formed and these interact with neutral sample molecules flowing through the discharge. Collisions between electrons and neutral sample molecule produces more sample ions. The newly produced ions collide frequently with other neutral molecules present to give thermolysed protonated ions like those produced by normal chemical ionisation. Thus, the yield of protonated ions from the standard APCI process is greatly increased (*Chemical Ionisation* and *Atmospheric Pressure Chemical Ionisation* are covered in the eponymous guides of Back-to-Basics). The corona discharge is relatively 'gentle' in that, at atmospheric pressure, it leads to more sample molecules being ionised without there being much fragmentation.

In inductively coupled plasmas, sample is introduced into a plasma struck in a flowing gas, frequently argon. The plasma itself is normally formed between the walls of two concentric cylinders so that the electric field has a non-uniform gradient. By applying a high frequency electromagnetic field across the plasma, ions and electrons are made to swing backwards and forwards as they attempt to follow

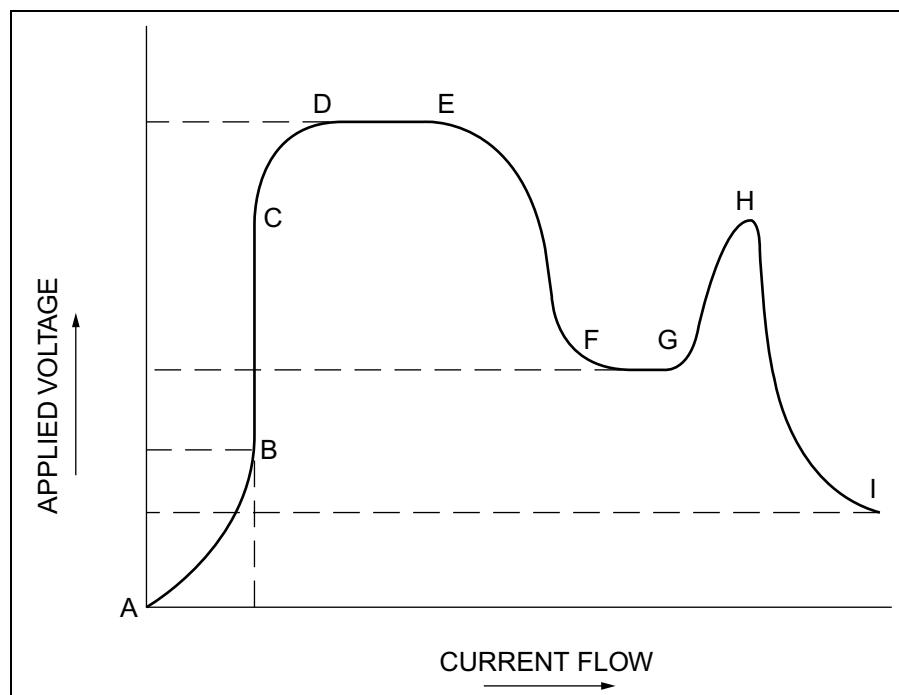


Figure 8. The graph shows the variation in current with changes in voltage between two electrodes placed in a gas. These variations in current flow are caused by changes in the flux of electrons passing between the electrodes inside the discharge chamber. An external resistance is used also to limit the total current that can pass. The variations are discussed in sections in the main text, labelled A to H on the diagram. Of particular note are the regions C–E (a corona discharge) and F–H (the start of the arc-forming process). The glow discharge and plasma occur mainly in sections E–F.

the changing alternating field. This effect leads to the ions and particularly the electrons being speeded up until they have energies equivalent to several thousand degrees. Under these conditions, any sample molecules in this plasma are rapidly degraded to atoms and ions of their constituent elements (see the Back-to-Basics guide, *Plasma Torch Ionisation Sources*). Unlike coronas, these inductively coupled plasmas are very destructive of sample so that the original structures are lost and only ions of the constituent elements are observed. This property makes them a valuable alternative to thermionic emission as ion sources for isotope ratio analysis.

With arcs, intense bombardment by ions and electrons and the heat produced at the electrodes causes sample molecules to be vaporised and broken down into their constituent elements. These sources are used particularly for analysis or isotope studies when the samples involved are inorganic, involatile and thermally very stable.

Effect of voltage changes on glow discharge characteristics

The glow discharge characteristics described above are typical of those found in a gas at reduced pressures but, as discussed, changes in gas pressure, the type of gas and the voltage applied all have important effects on the nature of the discharge, as the flow of electrons and ions in the discharge is affected by changes to these parameters. This section describes in greater detail the development of a discharge from a weak, non-selfsustaining state through to the corona/plasma/arc selfsustaining conditions.

The graph of Figure 8 illustrates the changes that occur in the electric current between two electrodes immersed in a gas as the voltage across them is increased. The circuit is completed by an external resistance, used to limit the current flow. As shown in Figure 8, the discharge can be considered in sections, which are described below.

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**Section A–B–C
(non-selfsustaining
discharge)**

This is the most difficult part of setting up a discharge because the discharge gases used are largely insulating and, theoretically, there are no electrons to start a current flow between the electrodes. However, there are sources of electrons and ions, some natural and some artificial:

(i) Background cosmic radiation.

Cosmic radiation consists of high speed charged particles, some of which interact with gas atoms in the discharge tube to produce electrons and ions. As might be imagined, this flow of electrons is spasmodic and not continuous but, under the right conditions of applied voltage and distance apart of electrodes, may be enough to initiate a continuous discharge.

(ii) Thermal emission

Application of an electric field between two metal electrodes causes a few ions and electrons to be desorbed. This is surface or thermal emission (see Back-to-Basics guide, *Thermal Ionisation (Surface Emission)*). Unless the electrodes are heated strongly, the number of electrons emitted is very small but, even at normal temperatures, it does add to the small number of electrons caused by cosmic radiation and is continuous.

(iii) Photoelectric effect

If photons of light of a suitable wavelength (usually UV or X-rays) impinge on a metal surface, electrons are emitted. This is the photoelectric (or photovoltaic) effect and can be used to start a flow of electrons in a discharge tube.

(iv) Piezoelectric spark

By use of a piezoelectric device, as in a gas-lighter, a small spark can be produced, which contains electrons and ions. If the spark is introduced into the gas in a discharge tube, it will provide the extra initial electrons and ions needed to start a continuous discharge. A plasma 'torch' is frequently 'lit' (started) in this fashion.

Given that some electrons and ions are present in the discharge gas from any of the processes described in (i)-(iv) above, the applied voltage causes the charged species to drift towards the respective positive and negative electrodes thereby constituting a small current flow. There is also another process, which is important, viz., some ions and electrons recombine to form neutral gas atoms again. Therefore, the electric current is the difference between the rate at which electrons and ions are produced and drift to the electrodes and the rate at which they disappear through electron/ion recombination (sometimes this occurs at the walls of the discharge vessel). As the voltage is increased, electrons and ions drift to the electrodes more rapidly and the current rises. This is the region A–B in Figure 8; at

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first, the relationship between the current flowing and the voltage applied is approximately in accord with Ohm's Law. However, as the voltage is increased, the current begins to rise less in accord with Ohm's law until, at an applied electric field of about 10 V/m, there is no further increase in current because insufficient numbers of electrons and ions are formed to offset the drift to the electrodes and recombination. At this point, increasing the voltage does not increase the current, which is said to be saturated. This is the straight section, B-C. The steady current is given by the equation, $i = \sqrt{q/r}$, in which q, r are respectively the rates at which electrons and ions are formed and then removed by recombination. This part of the discharge is not selfsustaining because stopping the initial production of electrons and ions ($q = 0$) leads to a shutdown in the total current flow (the discharge stops).

In the region, A-B-C, there is no light emitted from the discharge. At the electric field strengths used in this region, the discharge relies on the formation of ions and electrons by cosmic radiation, which is spasmodic process or thermal emission; the discharge is spasmodic and non-selfsustaining. Irradiating the cathode with UV light improves the flow of electrons, as does heating the electrode. If the electric field strength is made sufficiently high, even a spasmodic formation of electrons and ions may be enough to initiate a selfsustaining discharge (see below).

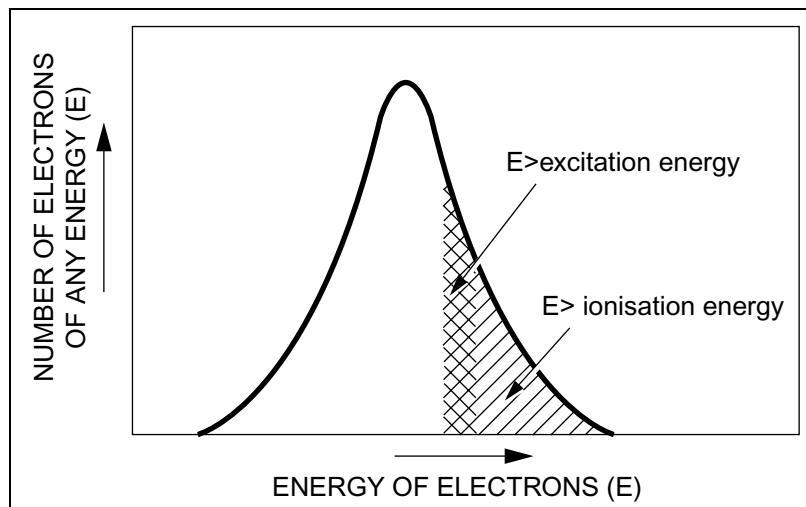


Figure 9. An idealised distribution of electron energies in an electron swarm drifting through a discharge gas. In this illustration, most electrons have not been accelerated sufficiently to cause any effect on neutral atoms on collision. These encounters between an atom and an electron are elastic. A narrow band of electrons has the right energy to excite electrons in an atom on collision ($E >$ excitation energy). In such an inelastic collision, the incident electron loses kinetic energy and an electron in the impacted atom is raised to a higher orbital level (Figure 8). When this electron drops back to a lower energy orbital, a photon of light is emitted; the atom remains neutral throughout. Above an electron energy exceeding the ionisation energy of a gas atom ($E >$ ionisation energy), an inelastic collision leads to an electron in the atom being ejected altogether, thereby leaving a positive ion. The extra electron produced in the collision causes an increase in the current flow in the discharge.

Section C–D–E

This is often referred to as the *Townsend breakdown region*, in which for little or no further change in voltage, the current may rise by several orders of magnitude, e.g., from 10^{-12} to 10^{-5} amperes. There is usually a spark produced during the initiation of this process. The current flow is controlled by the size of the resistance in the external ‘voltage’ circuit.

The drift of electrons towards the anode under the influence of an electric field is not a simple process. Electrons are accelerated by the electric field and gain kinetic energy ($mu^2 = 2eV$). As the electrons pick up speed, they collide with gas atoms and lose some of their kinetic energy through elastic collisions. The collision processes are such that some electrons are ‘knocked’ sideways, some are even reversed in direction and some are scarcely affected. Thus, it is necessary to think in terms of there being a spread in electron energies as the electrons drift towards the anode. In a complete vacuum, electrons would race in straight lines from the cathode to the anode at high speed but, in the discharge gas, the multiple collisions slow them down considerably. For example, in a vacuum, a fall of 100 volts would accelerate electrons to a speed of 4×10^7 cm/sec but their drift velocities in a discharge gas are only about 10^3 to 10^4 cm/sec, about a thousand times slower. The distribution of electron energies in a drift situation is approximately of the Boltzmann type, as shown in Figure 9. The electrons have a range of kinetic energies and are therefore at different temperatures.

Depending on the strength of the applied electric field, some electrons in the ‘swarm’ will have sufficient energy to cause ionisation of neutral gas atoms, viz., their energies are greater than the ionisation energy of the discharge gas and inelastic collisions between them and gas atoms form positive ions and more electrons (Figure 6). Thus, in this C–D region, as the external voltage is increased, more and more electrons will gain sufficient kinetic energy to cause ionisation of gas atoms. Since one incident electron in collision with a neutral gas atom leads to the production of two electrons (plus one positive ion) leaving the collision site, these inelastic collisions start a cascade process, whereby more and more electrons are formed as the swarm drifts down the discharge tube. The current flow, once initiated, becomes selfsustaining in that as many new electrons are produced as reach the anode and are discharged.

At first, this ionisation process only takes place near the anode, where some electrons will have gained sufficient energy to cause ionisation. However, as the discharge builds up (section D–E), the electric field gradient near the cathode becomes greater and greater due to the ionic space charge. Because of the steeper field gradient, electrons are

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accelerated more and more near the cathode and, therefore, ionisation through collision begins earlier and earlier in the space between the electrodes. The cascade process, by which electrons produce more electrons by ionisation of gas atoms and then these electrons produce even more electrons, gives rise to a sharp increase in current for no increase in voltage.

Section E–F–G

In the region (D–E), the resistance in the external circuit across the electrodes can be cut back because the selfsustaining process produces sufficient electrons and ions to maintain the discharge. Near E, the discharge gas begins to glow slightly. This is due to the fact that some electrons have gained just sufficient energy to cause excitation of atoms but not enough to cause ionisation (Figure 9). This ‘band’ of electron energies is relatively narrow so that there are relatively few exciting collisions and therefore the glow is initially fairly faint. This faint glow is sometimes referred to as the ‘subnormal’ glow. At this point, more and more electrons begin to be produced by other processes.

The extra sources of electrons that become important are known as secondary ionisation processes and are caused by, (i) bombardment of the cathode by incoming positive ions, which causes release of electrons, (ii) irradiation of the cathode by the glow that starts (photoelectric release of electrons) and, (iii) impact of excited atoms onto the cathode (similar to the electron release caused by ions).

As these extra sources of electrons become more important, the glow increases and, along section F–G, it becomes steady and similar to that shown in Figure 4 (this is the normal glow).

Section G–H–I

In the previous section (F–G), the glow only covers part of the cathode at F but the whole of the cathode at G. At this last point, the discharge has run out of efficient ways of generating electrons and for the current density to be maintained, more and more of the cathode is covered by the glow. The current density (= current flowing/area of cathode covered by the discharge = j) remains constant throughout the region F–G. If the voltage is now increased again the current rises slowly but, for a large increase in electron flow to be obtained, other ways of generating electrons have to be initiated. The glow begins to cover not only the cathode itself but also its supports and even the walls of the discharge tube (this is the region of the ‘abnormal’ glow). Eventually, new methods of producing electrons begin to be effective (H in Figure 8). At this point, the voltage may be reduced because an arc strikes between the electrodes. The earlier

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glow becomes concentrated into a very bright spot on the cathode surface and the positive column (plasma) glows brightly; the electric current flow increases by several orders of magnitude.

The new processes that cause this arcing to take place may be summarised as follows:

(i) Positive ions bombarding the cathode produce more and more electrons and cause the electrode to heat up. The heating causes thermal (surface) emission of electrons. For this process to be important, the temperature of the electrode needs to be high but this may lead to it melting if the melting point is too low or if the heat generated cannot be dissipated rapidly. Thus, for an arc struck with carbon electrodes, the high melting point of carbon leads to the electrodes becoming very hot and emitting a good supply of electrons, without melting. This is referred to as a hot discharge. Other arc discharges are 'cold' or relatively so. For low melting point electrode materials to produce an arc without melting, as with copper or mercury, an alternative process to thermal emission is necessary and this is discussed next.

(ii) In the arc discharge, the cathode and negative glows are so close to the cathode itself that their distances from it are very small, of the order of 10^{-7} m. This makes the electric field strength very high (field strength = voltage/distance) at about 10^8 to 10^9 volts/metre. If there are small imperfections (points, edges, corners having very small radii of curvature) on the surfaces of the electrodes, these field strengths will increase again, making field ionisation a new source of electrons (see Back-to-Basics, *Field Ionisation*). The arc from a cold discharge tends to wander over the cathode surface, rather as lightning tends to not to strike the same place twice but the arc from a hot discharge tends to remain anchored to one spot on the cathode surface.

In both low and high temperature arcs, the discharge begins as a very bright spot on the cathode. The area of the spot depends on the current flowing but the current density may reach 10^{10} amps/m². In the area of the spot, the temperature can reach about 2000 K for a hot discharge. At the anode, the temperature discharge is spread over the whole electrode and the temperature of the latter is accordingly lower.

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Overall process If, for any one gas, at any one pressure lying between two metal electrodes at any specified distance apart, a voltage be applied to the electrodes, a series of events begins, which is described as an electrical discharge. At low voltages, the discharge current is very small and is non-selfsustaining. At the breakdown region, a cascade of electrons is produced by collision processes between electrons and neutral gas atoms. This is the corona/plasma region, where the discharge becomes selfsustaining. As the voltage continues to be increased, atoms also become sufficiently excited as to emit light (a glow appears). With increasing voltage the glow moves nearer to the cathode and the positive column (the plasma) increases in length and glows more strongly, one end of it approaching the cathode, the other almost touching the anode. At even higher voltages, more ionisation processes begin and the current flow becomes very high, a bright spot appearing on the cathode. At this stage an arc has been struck. The various stages depend critically on the type of gas, its pressure and the configuration of the electrodes (their distance apart and their shapes control the size and shape of the applied electric field). By controlling the various parameters, the discharge may be made to operate as a corona, a plasma or an arc at atmospheric pressure. All three discharges may be used as ion sources in mass spectrometry.

Conclusion

Under suitable conditions of pressure and other factors, application of a suitable voltage between electrodes immersed in a gas causes a discharge of electric current through the gas. Different stages of the discharge are described as coronas, plasmas and arcs, which differ according the conditions of gas pressure and voltages under which they are produced. As well as emitting light, the various discharges contain both ions and electrons and may be used as ion sources in mass spectrometry.

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Back to Basics Section A: Ionization Processes

CHAPTER A7

THERMAL IONISATION (SURFACE EMISSION OF IONS)

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Quick Guide

- When a metal wire (filament) is heated in a vacuum, electrons are formed as a 'cloud' above the surface.
- The yield of electrons depends on the temperature of the filament and on the fundamental degree of difficulty in separating the electrons from the metal. The latter is measured in electron-volts as a work function, ϕ .
- The electrons may be accelerated from the filament by an anode, which has a positive potential with respect to the filament.
- If there is a different material (M) on the heated metal surface, M will be evaporated as the temperature increases. The ionization energy (ionization potential, I) of M is also measured in electron-volts.
- As well as the evaporation of neutrals from the sample, some positive ions (M^+) are also produced, the number depending on the temperature and the energy difference, $I-\phi$. This production of positive ions is known as surface or thermal ion emission. The m/z values of the ions are characteristic of the elements in the sample.
- These positive ions may be accelerated towards a cathode, which is held at a negative potential with respect to the filament.
- With a suitable arrangement of potentials with respect to the filament, it is then possible to obtain a flow of positive ions from any substance previously deposited on the filament.
- The flow of positive ions is normally passed into a suitable ion analyser in order to separate them according to m/z value. For this surface ion emission process, z is always equal to 1 and, therefore, $m/z = m$. The flow of ions at each m/z value generates an "ion current", which is used to measure the abundances of the ions.
- Different types of mass analyser may be used as, for example, quadrupoles.
- This thermal ionisation process requires filament temperatures of about 1000-2000 °C. At these temperatures, many substances, such as most organic compounds, are quickly broken down and so the ions produced are not representative of the structure of the original sample substance placed on the filament.

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- Ionisation energies (I) for most organic substances are substantially greater than filament work functions (ϕ) and therefore $I-\phi$ is positive (endothermic) and few positive ions are produced.
- Many inorganic substances are stable at the filament temperatures used or they are changed to simpler substances. The ionisation energies for "inorganic elements" are generally low so that $I-\phi$ is negative (exothermic). Inorganic samples produce good yields of positive ions characteristic of the elements present in the sample substance.
- Organics produce no useful positive ions but the ions produced by inorganic samples are remarkably free from "background" interference and the resulting mass spectra are relatively simple. The ion currents derived from the positive sample ions at each m/z value, being free from background ions, represent an accurate measure of the amount of each element.
- The advantages of producing mass spectra free from background interference, of the ability to regulate the flow of ions by altering the filament temperature and of the possibility for changing the material of the filament to give a work function matching ionisation energies, have led to the use of thermal ionisation for the precise measurement of isotope ratios in a variety of substrates.
- Thermal or surface emission of ions is one of the oldest ionisation techniques used for isotope ratio measurements.

Summary Heating inorganic substances to a high temperature on a metal filament gives characteristic positive ions, which may be mass analysed for m/z value and abundance so as to give accurate isotope ratios.

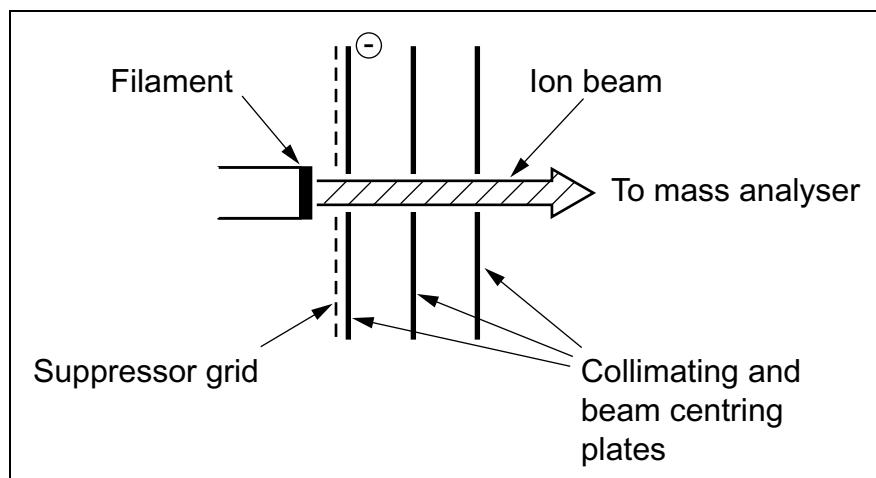


Figure I A typical filament assembly. Positive ions from the filament are accelerated by a high negative potential of about 1000 volts placed on the first collimating plate. The other plates are used for further collimation and centring of the ion beam, which is directed into a suitable mass analyser. Some positive ions strike the edge of the first collimating slit and produce secondary negative ions and electrons, which would be accelerated back onto the filament without a suppressor grid. This back-bombardment of the filament would lead to the formation of extraneous positive ions. To reduce this process to a minimum, a suppressor grid (at a potential of about -300 volts with respect to the filament) is included to deflect any back-scattered ions.

THERMAL IONISATION (SURFACE EMISSION OF IONS)

Introduction

It has been known for many years that heating a metal wire strongly in a vacuum causes emission of electrons from the metal surface. This effect is important for thermionic devices used for controlling or amplifying electrical current but this aspect of surface emission will not be considered here. Rather, the effect of heating a sample substance to a high temperature on a metal wire or ribbon is the subject of this *Back-to-Basics* article. Placing a sample of any substance onto the surface of a metal (a filament) and then heating it strongly by passing an electric current through the filament in a vacuum causes positive ions and neutral species to desorb from its surface. Because of the high temperatures involved, only certain elements are useful for construction of filaments. Typically platinum, rhenium, tungsten and tantalum are used because they are metallic and can be heated to temperatures of about 1000 to over 2000 °C without melting. A further important criterion for the filaments is that they should not readily react chemically with surrounding gas or with any sample placed on them. Since hot filaments are used in a high vacuum so as to facilitate evaporation and the manipulation of the emitted ions and neutrals, interaction of the filaments with air or background vapours is automatically reduced to a low level.

Measurement of the masses and abundances of any emitted positive ions is effected by mass spectrometry, particularly for obtaining precise isotope ratios. Few negative ions are produced and then only through secondary processes, which may be suppressed electrically. Negative ions are not considered here. Samples examined by surface emission are almost always inorganic species because, at the high temperatures involved, any organic material is seriously degraded (thermolysed) and will react with the filaments. At temperatures of 1000 to 2000 °C, most inorganic substances give positive ions without reacting with the typical filament elements listed above.

To obtain positive ions from a sample, it must come into contact with the filament. This may be done by directing a gas or vapour over the hot filament but, more usually, the sample is placed directly onto a cold filament, which is then inserted into the instrument and heated. The positive ions so produced are accelerated from the filament by use of a suitable nearby negative electrode and are then passed into a mass analyser so that their m/z values may be measured (Figure 1). The use of a suppressor grid in the ion source assembly reduces background ion effects to a very low level. Many types of mass analyser could be

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used but, since very high resolutions are normally not needed and the masses involved are quite low, the mass analyser can be a simple type such as a quadrupole

The "ion current" resulting from collection of the mass-separated ions provides a measure of the numbers of ions at each m/z value (the ion abundances). It may be noted that, for this ionisation method, all ions have only a single positive charge, *viz.*, $z = 1$ so that $m/z = m$ and therefore masses are obtained directly from the measured m/z values. Thus, after the thermal ionisation process, m/z values and abundances of ions are measured. The accurate measurement of relative ion abundances is used to give highly accurate isotope ratios. This aspect is developed more fully below.

Element	Ionization Energy (eV)
Aluminium	5.98
Calcium	6.11
Carbon	11.26
Caesium	3.89
Copper	7.72
Gold	9.22
Lanthanum	5.61
Lead	7.42
Lithium	5.39
Rubidium	4.18
Strontium	5.69
Thorium	6.95
Uranium	6.08

Figure 2 The table lists first ionisation energies (eV) for some commonly examined elements. Because only singly charged ions are produced by surface emission from a heated filament, only first ionisation energies are given, *viz.*, those for M^+ and not for higher ionisation states, in which more than one electron has been removed. Note that most elemental ionization energies fall in the range of about 3-12 eV.

Element	Work function (eV)	Melting point (K)
Platinum	6.2	2028
Rhenium	4.8	3440
Tantalum	4.2	3120
Tungsten	4.5	3640

Figure 3 Values of the average work function (ϕ , eV) for the commonly used filament metals. The melting points of the metals are also shown to give some guidance as to the maximum temperature, at which they can be used. Normally, this maximum would lie a few hundred degrees below the maximum so as to prevent sagging of the filament.

High filament temperatures

The high temperatures necessary for producing ions means that organic substances are rapidly vaporised (evaporated) and/or thermally destroyed and therefore this surface ionization technique is generally not used to investigate them. Inorganic substances are generally much more stable thermally but also much less volatile. Although an inorganic sample may be changed on heating as, for example, with the formation of calcium oxide from calcium carbonate, the "inorganic" or "metal" (elemental) parts of such samples are not destroyed. For example, if a sample of a caesium salt were to be examined, the anionic part of the sample might well be changed on the hot filament but the caesium atoms themselves would remain and would still be desorbed as Cs^+ . Sometimes, the desorbed ions appear as oxide or other species, as with GdO^+ .

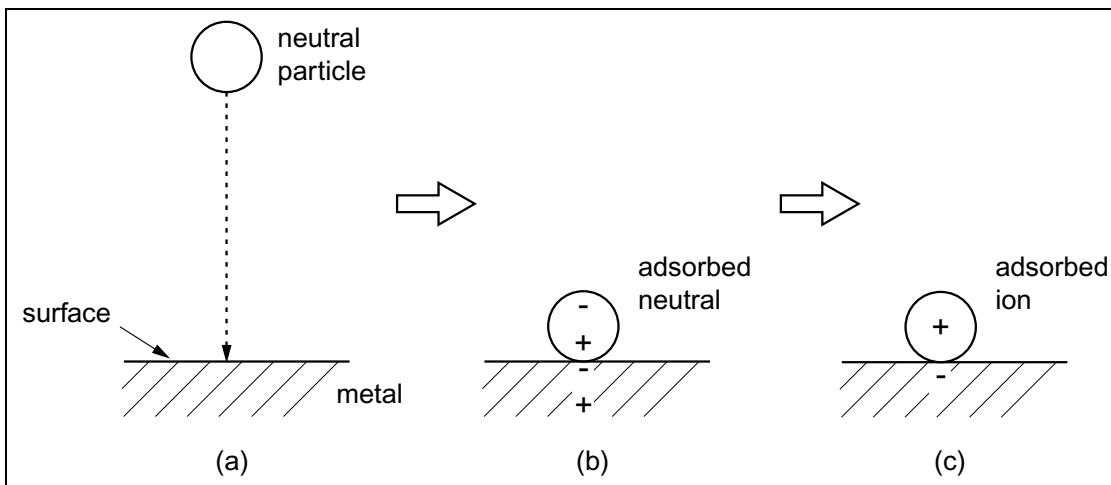
A further consequence of the high temperatures is that much of the sample is simply evaporated without producing isolated positive ions. There is a "competition" between formation of positive ions and the evaporation of neutral particles. Since the mass spectrometer examines only isolated charged species, it is important for maximum sensitivity that the ratio of positive ions to neutrals should be as large as possible. Equation (1) governing this ratio is given here.

$$\frac{n^+}{n^0} = Ae^{-(I - \phi)/kT} \quad (1)$$

In this equation, n^+/n^0 is the ratio of the number of positive ions to the number of neutrals evaporated at the same time from a hot surface at temperature T (K), where k is the Boltzman constant and A is another constant (often taken to be 0.5, and see below). By inserting a value for k and adjusting the expression (1) to common units (eV) and putting $A = 0.5$, the simpler equation (2) is obtained.

$$\frac{n^+}{n^0} = 0.5e^{11,600(\phi - I)/T} \quad (2)$$

The expression, $I-\phi$, in equation (1 or 2) is the difference between the ionisation energy (I ; sometimes known as the ionisation potential) of the element or neutral, from which the ions are formed and the work function (ϕ or sometimes W) of the metal, from which the filament is made. The ionisation energy and the work function control the energy needed to remove an electron from respectively a neutral atom of the sample and the material from which the filament is constructed. The difference between I and ϕ governs the ease with which positive ions can be formed from sample molecules lying on the filament. Both I and ϕ are positive and are frequently reported in units of electron-volts. Their importance is discussed in greater detail below. Some typical values for ionisation energies and work functions are given in Figure 2 and 3.

**Figure 4**

A schematic diagram showing the development of a dipolar field and ionisation on the surface of a metal filament.

(a) As a neutral atom or molecule approaches the surface of the metal, the negative electrons and positive nuclei of the neutral and metal attract each other, causing dipoles to be set up in each.

(b) When the neutral particle reaches the surface, it is attracted there by the dipolar field with an energy Q_a .

(c) As shown in the following figure, if the values of I and ϕ are apposite, an electron can leave the neutral completely and produce an ion on the surface and the heat of adsorption becomes Q_i . Similarly, an ion alighting on the surface may produce a neutral, depending on the values of I and ϕ . On a hot filament the relative numbers of ions and neutrals that desorb are given by equation I, which includes the difference, $I-\phi$, and the temperature, T .

Thermochemistry of surface emission

Adsorption of a neutrals (n^0) onto a metal surface leads to a heat of adsorption of Q_a , as the electrons and nuclei of the neutral and metal attract or repel each other. Partial positive and negative charges are induced on each with the formation of a dipolar field (Figure 4). Similarly, adsorption of ions (n^+) onto a metal surface leads to a heat of adsorption of Q_i . Generally, Q_i is about 2-3 eV and is greater than Q_a , which itself is about 1 eV. The difference between Q_i and Q_a is the energy required to ionise neutrals (n^0) on a metal surface so as to give ions (n^+) or vice versa. This difference, $Q_i - Q_a$, may be equal to, greater than or less than the difference, $I-\phi$, between the ionisation energy (I) of the neutral and the ease with which a metal can donate or accept an electron (the work function, ϕ). Where $Q_i - Q_a > I-\phi$, the adsorbed particle will be an ion, no matter whether it was originally a neutral particle or an ion that approached the metal surface. Similarly, if $Q_i - Q_a < I-\phi$, the adsorbed particle will be a neutral species, no matter whether it was an ion or a neutral that approached the metal surface. If energy is now added to the system by strongly heating the filament, desorption of ions and neutrals occurs. Clearly, the numbers of desorbing neutral particles and ions must depend on the size and sign of the difference in ($I-\phi$) and on the added energy, which is controlled by the absolute temperature, T . As shown in Figure 5, the critical value for desorption of ions and neutrals at any given temperature is governed by the relation, $Q_a = Q_i - (I-\phi)$. This is the criterion used in the derivation of equation I.

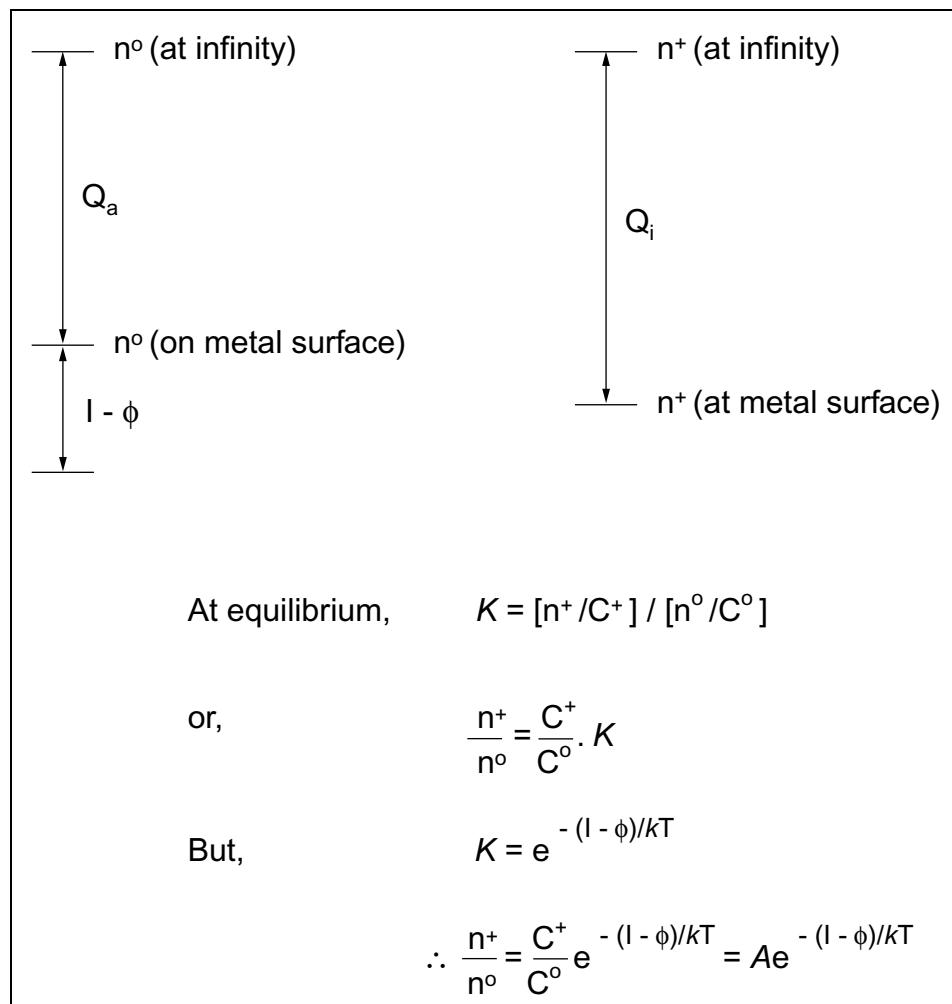


Figure 5 On bringing a neutral (n^o) from infinity to the surface of a filament metal, it adsorbs with an energy Q_a (Figure 4). Similarly, bringing an ion (n^+) from infinity leads to a heat of adsorption of Q_i . If the difference between Q_a and Q_i is equal to $I-\phi$, the neutral particle may desorb again as a neutral or may desorb as an ion, depending on the value of $I-\phi$. Similar arguments apply to an adsorbed ion (n^+), which may desorb again as an ion or may desorb as a neutral.

On the surface of a heated filament metal, no matter whether ions or neutrals are adsorbed initially, an equilibrium will be set up between them, with equilibrium constant K (shown in Figure 5). For the equilibrium constant, surface "concentrations" of desorbing neutrals (n^0) and ions (n^+) must be used. The surface "concentration" of ions is the proportion of ions actually desorbing to the total number on the surface (C^+), viz., the concentration of desorbing ions = $[n^+ / C^+]$. Similarly, the concentration of desorbing neutral particles = $[n^0 / C^0]$ (Figure 5). In the well-known thermodynamic equation that governs an equilibrium process, $\ln K = -\Delta G/RT$, the gas constant (R) can be replaced by its equivalent (the Boltzmann constant, k) and the total free energy change (ΔG) by the critical energy change ($I-\phi$). From these substitutions, the expression, $K = \exp(-(I-\phi)/kT)$, is obtained. Combining this with the expression for K shown in Figure 5, equation I is revealed. Therefore, the ratio of the number of ions to the number of neutrals desorbing from a heated filament depends not only on the absolute temperature but also on the actual surface coverage of ions and neutrals on the filament (C^+, C^0) and crucially on the difference between the ionisation energy and work function terms, I and ϕ . This effect is explored in greater detail in the following illustrations.

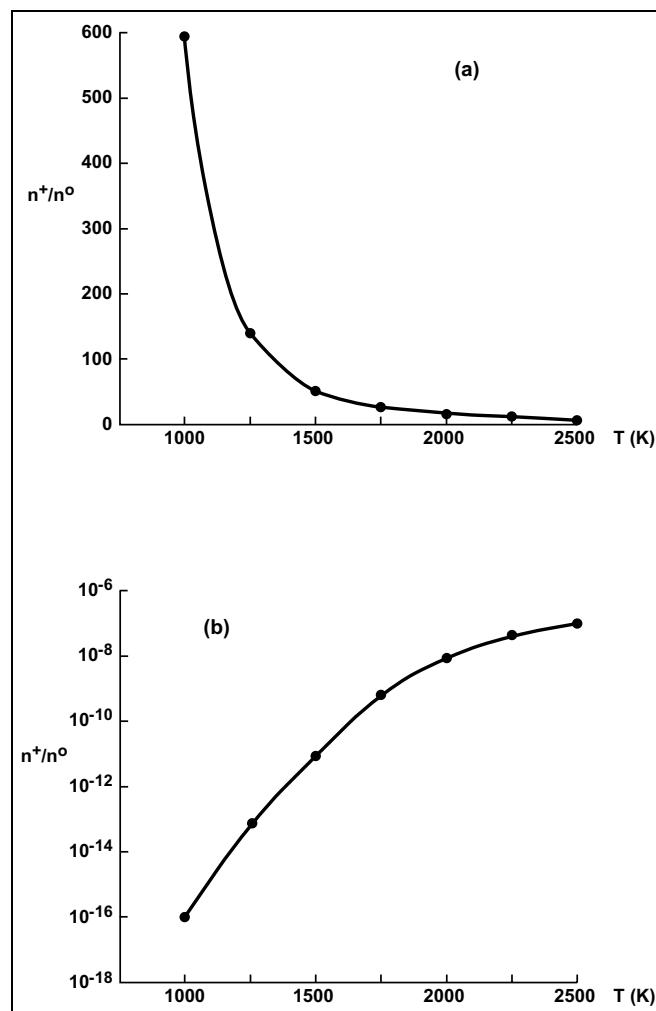


Figure 6 Graphs showing the influence of work function and ionisation energy on the efficiency of ionisation. Using equation (1), the ratio n^+ / n° was calculated for uranium ($I = 6.08$ eV) on either (a) a platinum filament ($\phi = 6.2$ eV) or (b) a rhenium filament ($\phi = 4.8$ eV), at different temperatures. For platinum (a), a good yield of ions is obtained but the ratio n^+ / n° falls with increasing temperature. For rhenium (b), the relative ion yield is small but increases with increasing temperature. The highest ion yields are given by the use of platinum with uranium, for which $I - \phi$ is negative by about 0.1 eV.

Both of the terms I and ϕ have positive values. Examination of equation (1 or 2) reveals that, for $\phi > I$, then $\phi - I$ is positive and the greater the temperature, the smaller the proportion of positive ions to neutrals. For example, with a sample of caesium (ionisation energy, 3.89 eV) on a tungsten filament (work function, 4.5 eV) at 1000 K, the ratio of $n^+ / n^0 = 591$. Thus, for every caesium atom vaporised, some 600 atoms of Cs^+ ions are produced. At 2000 K, the ratio of n^+ / n^0 becomes 17 and only about 20 ions of caesium are evaporated for every Cs atom (Figure 6a). For $\phi < I$, as with lead ($I = 7.42$ eV) on tantalum ($\phi = 4.2$ eV), the corresponding figures are 3×10^{-16} at 1000 K and $I \times 10^{-8}$ at 2000 K (Figure 6b). Clearly, the lower the ionisation energy with respect to the work function, the greater the proportion of ions to neutrals produced and the more sensitive the method. For this reason, filaments are used, for which their work functions give the best yields of ions. The evaporated neutrals are lost to the vacuum system. With continued evaporation of ions and neutrals, eventually no more material remains on the filament and the ion current falls to zero.

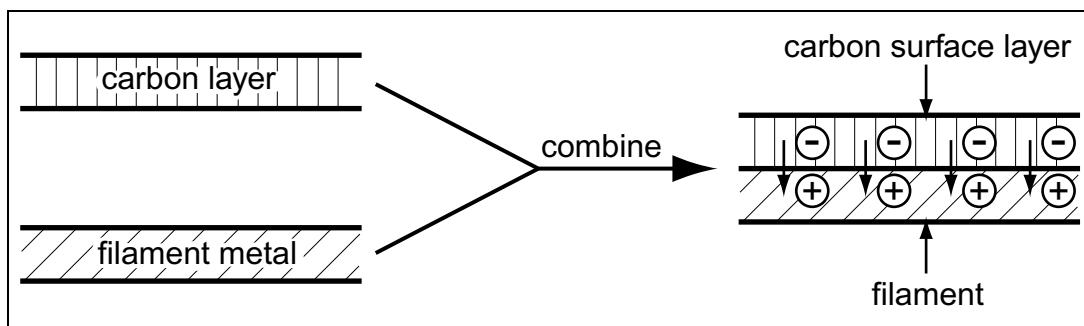


Figure 7 Schematic diagram showing how placing a thin layer of highly dispersed carbon onto the surface of a metal filament leads to an induced dipolar field having positive and negative image charges. The positive side is always on the metal, which is much less electronegative than carbon. This positive charge makes it much more difficult to remove electrons from the metal surface. The higher the value of a work function, the more difficult it is to remove an electron. Effectively, the layer of carbon increases the work function of the filament metal. Very finely divided silicon dioxide may be used in place of carbon.

Changing the work function (activators)

For an element of ionization energy I , equation (2) shows that, at any one temperature, the work function of the surface from which emission of particles occurs is clearly crucial to the proportion of ions produced in relation to the number of neutrals. As $I-\phi$ changes from negative to positive, the ion yield becomes progressively smaller. Figure 3 indicates that platinum would be the filament metal of choice in most applications because it has the biggest work function of the four metals commonly used (Pt, Re, W or Ta). However, platinum also has the lowest melting point and to reach the high temperatures needed to effect suitable evaporation rates, it may be necessary to use a metal such as tantalum or rhenium, for which the work functions are smaller. Thus, there must be a "trade-off" between work function and temperature in order to maximise ion yield. For difficult cases, this dilemma may be solved by using "activators" on the surface of the filaments. The activators commonly used are colloidal or very finely dispersed (high surface area) silicon dioxide or carbon. These substances are much more electronegative than the filament metal and produce a dipolar field (Figure 7). This field induces a positive image charge in the filament surface, thereby making removal of electrons more difficult and increasing its effective work function. Since ϕ is increased, the difference $I-\phi$ must change and therefore the ion yield ratio, n^+/n^0 . Activators are used to improve ion yield when examining metals of high ionization energy, as with uranium, lead or plutonium on tungsten filaments.

Amount of sample

The rate of evaporation of ions from a heated surface is given by equation (3), in which Q_i is the energy of adsorption of ions on the filament surface (usually about 2-3 eV) and C_i is the surface density of ions on the surface (a complete monolayer of ions on a filament surface would have a surface density of about 10^{15} ions/cm $^{-2}$).

$$n^+ = C_i e^{-Qi/kT} \quad (3)$$

Similarly, the rate of evaporation of neutral species from a filament surface is given by equation (4), in which C_o is the surface density of atoms on the surface (a complete monolayer of atoms would have a surface density of about 10^{15} atoms/cm $^{-2}$). .

$$n^0 = C_o e^{-(Qi - f + l)/kT} \quad (4)$$

Dividing equation (3) by (4) yields equation (1), in which $A = C_i/C_o$.

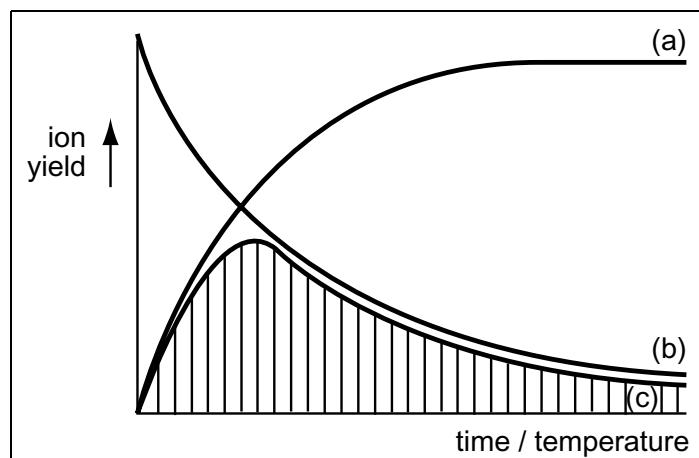


Figure 8 Graph (a) illustrates the effect of increasing temperature on the ion yield, which increases as the temperature rises. Graph (b) shows the effect of decreasing surface coverage of the filament surface as ions and neutrals evaporate; as the surface densities of ions and neutrals decrease, the ion yield falls off with time. Graph (c) gives an example of the shape of curve resulting from the two effects. As the temperature of the surface is increased to improve ion yield, the surface is depleted of sample more and more rapidly until no sample remains and therefore no ion current. The area under curve (c) represents the total ion yield.

As ions and neutrals evaporate from a heated filament surface the amount of sample decreases and the surface densities (C_i , C_o) must decrease. Therefore, equation (1) covers two effects. The first was discussed above and concerns the changing value for the ratio n^+/n^o as the temperature of the filament is varied and the other concerns the change in the total number of ions desorbing as the sample is used up. The two separate effects are shown in Figure 8a,b. Combining the two effects (Figure 8c) reveals that, if the temperature is increased to maintain the flow of ions, which drops naturally as the sample is used up (time), then eventually the flow of ions and neutrals becomes zero whatever the temperature of the filament because the sample runs out (it has all disappeared from the filament surface).

Measurement of ratios of isotopic abundances

For any one ion type (e.g., Cs^+), measurement of its abundance in a sample requires the sample to be evaporated over a period of time. The total yield of ions is obtained by integrating the area under the ion yield curve (Figure 8c).

Generally, ratios of isotopic abundances need to be obtained and not individual total ion yields. Experimentally, for two isotopes M_1 and M_2 , this entails the simultaneous measurement of their abundances as given by the ion current for the two masses arriving at the ion collector. For two isotopes, the ion yields are given by equations (5,6), which are obtained simply from equation (1) by inserting the relevant values for C_i , C_o and Q . Not only are C_i and C_o different (because the relative amounts of isotopes are different) but they vary with time, as discussed above. Dividing equation (5) by (6) gives equation (7), from which it is clear that, at any one temperature, since the ratio of C_1 to C_2 changes with time, then the ratio of ion yields for isotopes M_1 and M_2 must change with time.

$$\frac{n_1^+}{n_1^o} = C_1 e^{-(Q_1 - \phi + I_1)/kT} \quad (5)$$

$$\frac{n_2^+}{n_2^o} = C_2 e^{-(Q_2 - \phi + I_2)/kT} \quad (6)$$

$$\frac{[n_1^+/n_1^o]}{[n_2^+/n_2^o]} = \frac{C_1}{C_2} e^{-(\Delta Q + \Delta I)/kT} \quad (7)$$

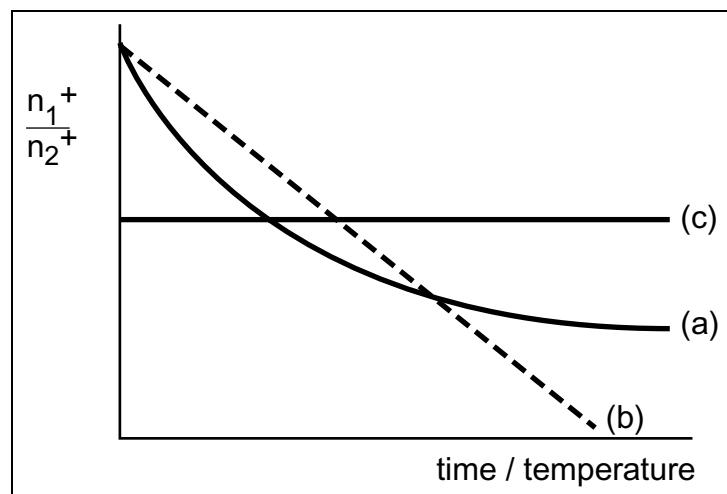


Figure 9 Schematic illustrations of the effect of temperature and surface density (time) on the ratio of two isotopes. Graph (a) shows that, generally, there is a fractionation of the two isotopes as time and temperature change, *viz.*, the ratio of the two isotopes changes throughout the experiment and makes difficult an assessment of their precise ratio in the original sample. Graph (b) illustrates the effect of gradually changing the temperature of the filament to keep the ratio of ion yields linear; this makes estimation of the ratio in the original sample much easier. The best method is one in which the rate of evaporation is low enough that the ratio of the isotopes is virtually constant; this ratio then relates exactly to the ratio in the original sample.

Figure 9 shows a schematic representation of this effect, in which the ratio of the two isotopes changes with time. To obtain an accurate estimate of the ratio of ion abundances, it is better if the relative ion yields decrease linearly (Figure 9). This may be effected by adjusting the filament temperature continuously so as to obtain the linear response required. An almost constant response for the isotope ratio may be obtained by slow evaporation of the sample, *viz.*, by keeping the filament temperature as low as is consistent with sufficient sensitivity of detection (Figure 9).

It may be seen from the above description that measurement of precise isotope ratios requires a substantial amount of operator experience, particularly with samples that have not been examined previously. A choice of filament metal must be made, the preparation of the sample on the surface is important (particularly when activators are used) and the rate of evaporation and therefore temperature control may be crucial. However, this method of surface ionisation is used to measure precise isotope ratios for multiple isotopes. Other *Back-to-Basics* articles discuss practical details and applications.

Conclusion

Precise isotope ratio measurements can be made by comparing the yields of isotopic ions desorbing from a sample placed on a strongly heated filament, which is generally made from platinum, tantalum, rhenium or tungsten.

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**Back to Basics Section B:
Interfaces and Ionization Techniques**

CHAPTER B1

ELECTROSPRAY IONIZATION (ES)

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Quick Guide

- Electrospray is both an atmospheric pressure (API) liquid inlet system for a mass spectrometer and, at the same time, it is an ionization source.
- Electrospray uses an electric field to produce a spray of fine droplets.
- In many applications of mass spectrometry it is necessary to obtain a mass spectrum from a sample dissolved in a solvent. The solution cannot be passed straight into the mass spectrometer because, in the high vacuum, the rapidly vaporizing solvent would entail a large pressure increase causing the instrument to shut down.
- Therefore, the sample solution, which may or may not come from a liquid chromatographic column, is passed along a narrow capillary tube, the end of which is maintained at a high positive or negative potential.
- The high potential and small radius of curvature at the end of the capillary tube creates a strong electric field which causes the emerging liquid to leave the end of the capillary as a mist of fine droplets mixed with vapour. This process is nebulization and occurs at atmospheric pressure.
- The droplets, which carry positive or negative charges depending on the sign of the applied potential, pass into and along a small evaporation chamber. Much of the excess of solvent vapour is allowed to flow to atmosphere or may be gently exhausted to waste.
- As the droplets pass through the chamber, they evaporate and rapidly become much smaller through vaporization of solvent. At the same time, because the surface area of the droplets gets smaller and smaller, the density of electrical charge on the surface increases until a point of instability is reached.
- Eventually, not just neutral solvent molecules but also ions start to desorb from the surface of each droplet.
- Ions, residual droplets and vapour formed by electrospray are extracted through a small hole into two more evaporation chambers (evacuated) via a 'nozzle' and a 'skimmer' and then pass into the analyser of the mass spectrometer where a mass spectrum of the original sample is obtained.

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- This inlet/ion source is a simple system with no moving parts and yields many ions from the original dissolved sample. Even more attractive is the tendency for electrospray to produce multicharged ions, a benefit that makes accurate measurement of large relative molecular masses much easier.
- When multicharged ions are formed, the simple rule of thumb used widely in mass spectrometry that $m/z = m$ because usually, $z = 1$, no longer applies; for $z > 1$ then $m/z < m$ and the apparent mass of an ion is much smaller than its true mass. Accurate mass measurement is much easier at low mass than at high and the small m/z values, corresponding to high mass with multiple charges, yield accurate figures for the high mass.
- Electrospray can be used with both sector, time-of-flight and quadrupole instruments. The technique has been used extensively to couple liquid chromatographs to mass spectrometers.

Summary

A sample to be examined by electrospray is passed as a solution in a solvent (made up separately or issuing from a liquid chromatographic column) through a capillary tube held at high electrical potential so that the solution vaporizes and emerges as a spray or mist of droplets (it is nebulized). As the droplets evaporate, residual sample ions are extracted into a mass spectrometer for analysis.

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ELECTROSPRAY IONIZATION

Introduction

For a more detailed description of the ionization process inherent in electrospray, please consult 'Atmospheric Pressure Ionization' in these Back-to-Basics Guides. The reader should compare electrospray with thermospray (see relevant Guide).

In many applications in mass spectrometry the sample to be analysed is present as a solution in a solvent which could be, for example, organic as with methanol or acetonitrile or aqueous, as with body fluids. The solution could also be an effluent from a liquid chromatography column. In any case, a solution must flow into the 'front end' of a mass spectrometer but, before it can provide a mass spectrum, the bulk of the solvent must be removed without losing the sample (solute). If the solvent were not removed then its vaporization as it entered the vacuum of the mass spectrometer would produce a large increase in pressure and stop the instrument from working. At the same time that this excess of solvent is removed, the dissolved sample must be retained so that its mass spectrum can be measured, viz., there must be differentiation between solvent and solute (sample) molecules. There are several means of effecting this differentiation between carrier solvent and the solute of interest, and electrospray is just one of them. However, there is an additional important consideration in electrospray. Unlike the other methods of introducing a liquid into a mass spectrometer, electrospray frequently produces multicharged ions which make accurate large mass measurement easier and gives this inlet/ion source a considerable advantage in areas such as peptide and protein research (see below).

One of the first successful techniques for selectively removing solvent from a solution without losing the dissolved solute was to add the solution dropwise to a moving continuous belt. The drops of solution on the belt were heated sufficiently to evaporate the solvent and the residual solute on the belt was carried on into a normal EI or CI ion source where it was heated more strongly so that it in turn volatilized and could be ionized. The moving belt system had some mechanical problems and could be temperamental. The more recent, less mechanical inlets such as electrospray have displaced it.

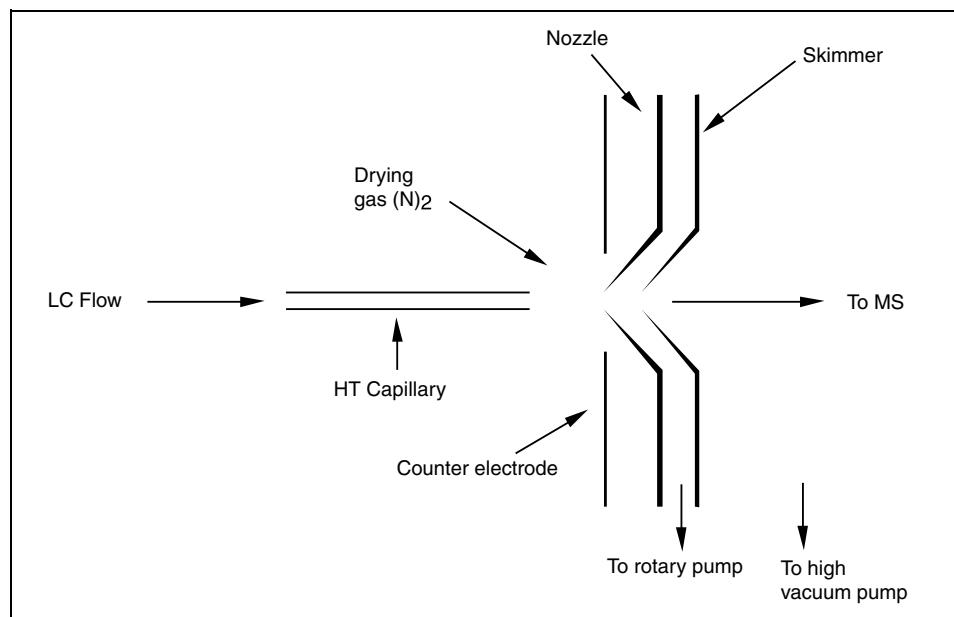


Figure I Schematic diagram of an electrospray inlet/ion source. A spray produced from the high electrical voltage (HT) on the capillary moves towards a hole in the electrical counter-electrode. After removal of much solvent, sample ions continue under their momentum through the hole and then through the nozzle and skimmer, where most remaining solvent is removed.

Differential Solvent Removal

A sample for which a mass spectrum is required may well be dissolved in an organic or an aqueous solvent. For example, in searching for drugs in blood plasma, the plasma itself may be investigated (aqueous) or its active components may be first extracted into an organic solvent such as dichloromethane. Alternatively, the sample may first be separated into its components by passage through a liquid chromatographic instrument (see Back to Basics Guide on LC); on emerging from the column the sample of interest is present as a solution in the solvents used in the chromatography. In either case, the sample to be examined is in solution and cannot be put straight into a mass spectrometer without first removing most of the solvent and without, of course, removing the dissolved sample also!

Electrospray is one method for effecting this differential solvent removal. The solution is passed along a short length of stainless steel capillary tube, to the end of which is applied a high positive or negative electric potential, typically 3-5 kV (Figure 1). When the solution reaches the end of the tube, the powerful electric field causes it to be almost instantaneously vaporized (nebulized) into a jet or spray of very small droplets of solution in solvent vapour. Before entering the mass spectrometer proper this mist of droplets flows through an evaporation chamber which is heated slightly to prevent condensation. As the droplets move through this region, solvent evaporates rapidly from their surfaces and the droplets get smaller and smaller. In addition to producing the spray, this method of rapid vaporization leaves no time for equilibrium to be attained and a substantial proportion of the droplets have an excess of positive or negative electrical charge which resides on their surfaces. Thus, as the droplets get smaller the electrical surface charge density increases until such time that the natural repulsion between like charges causes ions as well as neutral molecules to be released. The end of the capillary tube is aimed at a small hole (target) at the opposite end of this evaporation region. After vaporizing from the surface of a droplet, solvent molecules which have low molecular mass quickly diffuse away from the 'line-of-sight'. A Z-spray ion source operates slightly differently (see Z-spray guide).

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However, the sample molecular ions and cluster ions have much greater molecular mass (and therefore momentum) than those of the solvent and tend to carry straight on towards the target at the end of the inlet region (Figure 1). To assist evaporation of the droplets and the breaking up of unwanted cluster ions, a drying gas (nitrogen) flows along and past the end of the capillary (Figure 1). If the gas is arranged to flow between the counter electrode and the nozzle, it is sometimes referred to as a ‘curtain’ gas. At the ‘target’ hole, the heavier ions pass through but most of the lighter solvent molecules have by this time diffused away and do not pass through. In effect the device is a momentum separator between solvent and solute (sample) molecules. After passing through this hole, the ions pass through two evacuated regions via a nozzle and a skimmer (Figure 1). These conically shaped holes refine the separation of sample ions from solvent ions, still mainly on the basis of momentum but also by an extraction and focusing effect of electrical potentials applied to the nozzle and skimmer. Finally, sample ions pass into the analyser of the mass spectrometer where their mass-to-charge (m/z) ratios are measured in the usual way. The mass spectrometer may be of any type.

The end result of the above process means that sample molecules dissolved in a solvent have been extracted from the solvent and turned into ions. Therefore, the system is both an inlet and an ion source and a separate ion source is not necessary.

The ions passing into the mass spectrometer analyser from electrospray have little excess of internal energy and therefore not enough energy to fragment. Many of the ions are of the form $(M+X)^+$ or $(M-H)^-$ in which X may be hydrogen or some other element such as sodium or potassium. Whilst these ‘quasimolecular’ ions are excellent for giving accurate molecular mass information, and this may be all that is required, they give little or none of the information concerning the actual molecular structure of the substance being investigated, which is provided by fragment ions. This is entirely analogous to the problem with simple chemical ionization and similar solutions to it exist. To give the quasimolecular ions the extra energy needed for them to fragment, they may be passed through a ‘collision gas’ and the resulting spectra analysed for metastable ions (MS/MS methods). An alternative arrangement uses the potential difference between the electrodes (cone voltage) to accelerate the ions. If the voltage difference is increased, collisions between the faster moving ions and neutral molecules leads to fragmentation, as in CI. If the cone voltage is reduced, the ions slow down and resulting collisions have insufficient energy for fragmentation.

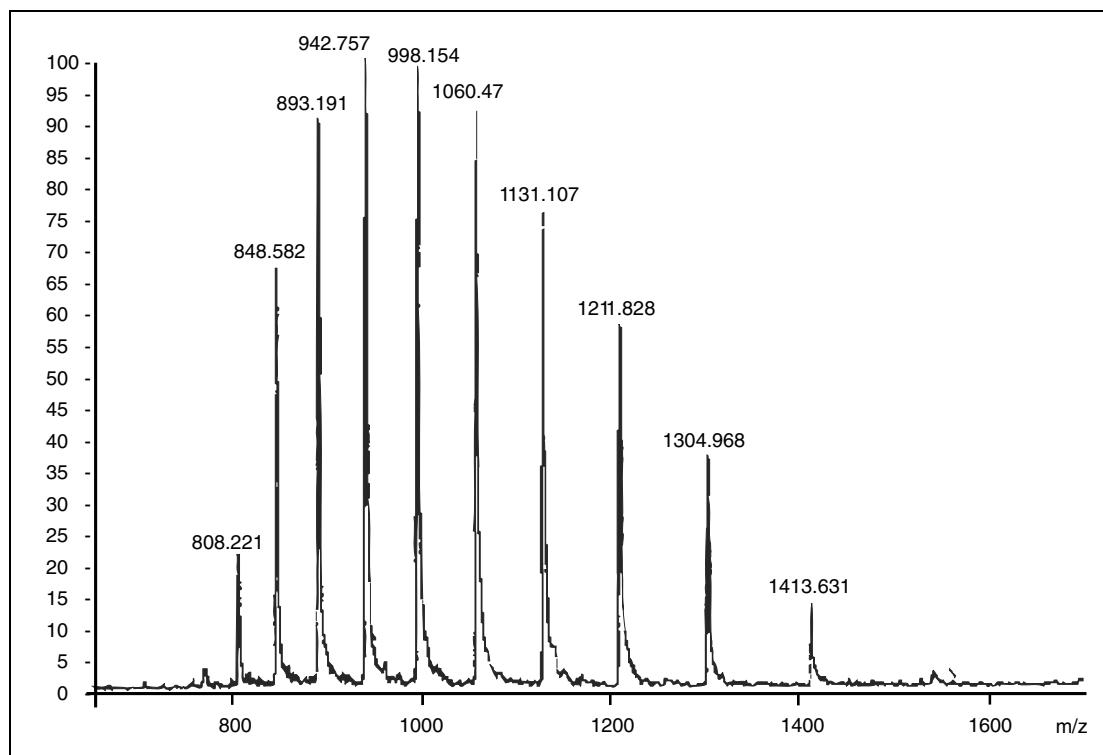


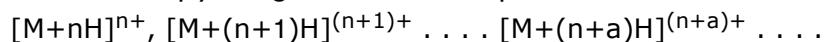
Figure 2 Quasimolecular ions, $[M+n.H]^{n+}$, from a protein (myoglobin) of molecular mass 16,951.5 daltons. In this case, n ranges from 21 (giving a measured mass of 808.221) to 12 (corresponding to a measured mass of 1413.631). The peaks with measured masses in between these correspond to the other values of n between 12 and 21. By taking successive pairs of measured masses, the relative molecular mass of the myoglobin can be calculated very accurately as shown in Figure 4.

Multiply-charged ions

There is another type of ion formed almost uniquely by the electrospray inlet/ion source and which makes this technique so valuable for examination of substances such as proteins, which have large relative molecular mass. Measurement of m/z ratios usually gives a direct measure of mass because, for most commonly experienced mass spectrometry, $z = 1$ and so $m/z = m/1 = m$. Values of z greater than one are unusual. However, for electrospray, values of z greater than one, often much greater, are quite commonplace. For example, instead of the $[M+H]^+$ ions common in simple CI, ions in electrospray can be $[M+nH]^{n+}$ where n may be anything from 1 to about 30.

Thus the m/z value for such ions is $[M+nH]/n$, if the mass of hydrogen is taken to be one. As a particular example, suppose $M = 10,000$. Under straightforward CI conditions, $[M+H]^+$ ions will give an m/z value of $10,001/1 = 10,001$, a mass which is difficult to measure with any accuracy. In electrospray, the sample substance may be associated with, say, 20 hydrogens. Now, the ion has a mass-to-charge ratio of $[M+20H]^{20+}$ and therefore $m/z = 10,020/20 = 501$. This mass is easy to measure accurately with a wide range of instruments.

Normally, a range of values for n is found, each molecule (M) giving a series of multiply-charged ions. For example, a series:



might be observed, each successive quasimolecular ion having one more hydrogen and one more electrical charge than the preceding one (figure 2). The only difficulty lies in knowing the value of n ! Fortunately this value is relatively easy to extract from the mass spectrum (figure 3). The derivation has been fully automated to such an extent that the complex of quasimolecular ions ($m_1, m_2 \dots m_r$) measured as shown in figure 3 can now be presented simply by the data system as a transformed spectrum with one molecular ion, M . An example of this is illustrated in figure 2 where the electrospray mass spectrum of haemoglobin, containing nine main measured masses, transforms into a much simpler looking spectrum having two main molecular ions (M_1 and M_2) arising from its alpha and beta chains; close inspection also reveals the molecular ion of the gamma chain.

Let the unknown mass be M and let the number of unknown charges be n, corresponding to the addition of n protons ($n.H^+$):

For two successive measured mass-to-charge ratios m_1 and m_2 , two equations can be written,

$$m_1 = (M+n)/n \quad (1)$$

$$m_2 = (M+n+1)/(n+1) \quad (2)$$

assuming a value of 1 for the atomic mass of hydrogen.

Equations (1) and (2) are simultaneous and can be solved easily to give M and n.

For a set of successive measured mass-to-charge ratios m_1, m_2, \dots, m_r , any successive pair can be chosen to calculate M. The best value for M will be obtained by averaging all the values individually calculated from all successive pairs. Thus, if m_1 and m_2 gave M' and m_2 and m_3 gave M'' , a best value for M would be $M = (M'+M'')/2$. Clearly, by averaging a lot of pairs a more accurate value for M can be found.

Figure 3 Calculation of true mass (M) from measured mass-to-charge ratios m_1, m_2, \dots, m_r .

Uses of Electrospray

Although simple solutions can be examined by these electrospray techniques, it is often the case that, for a single substance dissolved in a solvent, straightforward evaporation of the solvent outside the mass spectrometer with separate insertion of the sample is sufficient. This is not true for all substances. Peptides, proteins, nucleotides, sugars, carbohydrates, mass organometallics and many polar substances once isolated from solution as solids are then quite difficult to vaporize in a standard EI or CI ion source without thermally damaging them. For such substances it is best to leave them in solution and obtain their electrospray mass spectra. Electrospray ionization is convenient for most classes of compound and it is wrong to think of the technique as just an interface for coupling LC to MS. It can be used equally well for single substances by first dissolving them in a suitable solvent and then passing the solution into the electrospray inlet.

The ability of electrospray to accurately mass measure very large molecules makes it extremely valuable for a wide variety of applications, particularly biochemical and medical.

For mixtures the picture is different. Unless the mixture is to be examined by MS/MS methods, usually it will be necessary to separate it into its individual components. This is most often done by gas or liquid chromatography. In the latter, small quantities of emerging mixture components dissolved in elution solvent would be laborious to deal with if each component had to be first isolated by evaporation of solvent before its introduction into the mass spectrometer. In such circumstances, the direct introduction, removal of solvent and ionization provided by electrospray is a boon and puts LC/MS on a level with GC/MS for mixture analysis. Further, GC is normally concerned with volatile, relatively small molecular weight compounds and is of little or no use for the many polar, water soluble, high molecular mass substances such as the peptides, proteins, carbohydrates, nucleotides and similar found in biological systems. LC/MS with an electrospray interface is used frequently in biochemical research and medical analysis.

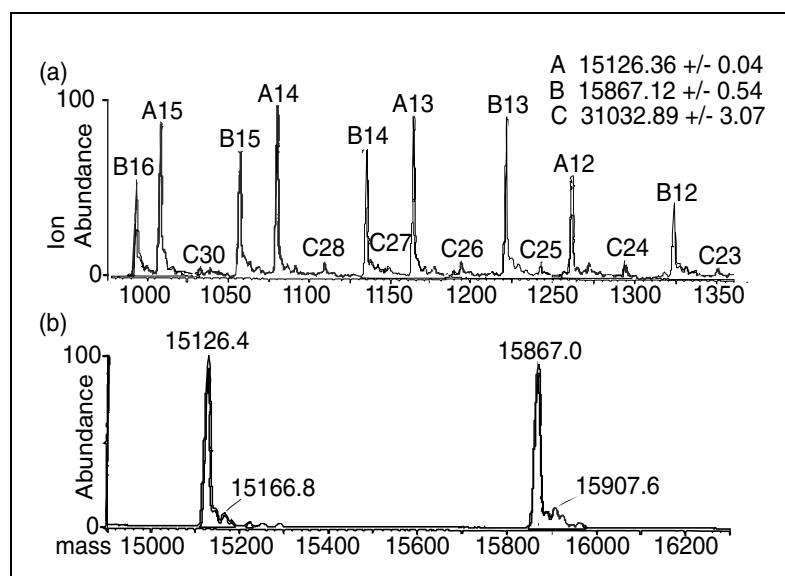


figure 4 Positive ion electrospray mass spectrum of human haemoglobin, (a) as initially obtained with all the measured masses. (b) After calculation of true mass, as in figure 3, the spectrum transforms into two main peaks representing the alpha and beta chains of haemoglobin with accurate masses as given. This transformation is fully automated. The letters A, B, C refer to the three chains. Thus, A13 means the alpha chain with 13 protons added.

- General Comments** Electrospray alone is a reasonably sensitive technique for use with many classes of compounds. Spectacular, unprecedented results have been obtained with accurate mass measurement of high molecular mass proteins but this should not be allowed to obscure the fact that electrospray is equally well suitable for much smaller molecules and for use as a routine atmospheric pressure inlet/ionization system for a mass spectrometer.
- Conclusion** By nebulizing a solution by a strong electric field, a spray of small charged droplets is produced from which the solvent can be removed, leaving sample ions to pass straight into the analyser region of a mass spectrometer. By producing multiply charged ions, electrospray is extremely useful for accurate mass measurement, particularly for thermally labile, high molecular mass substances.

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**Back to Basics Section B:
Interfaces and Ionization Techniques**

CHAPTER B2

ATMOSPHERIC PRESSURE IONIZATION (API)

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Quick Guide

- Under suitable conditions, a stream of solvent containing a substrate dissolved in it can be broken up into a spray of fine droplets at atmospheric pressure (*nebulized*).
- Some of the droplets carry an excess of positive electric charge and others an excess of negative electric charge.
- The spray or stream of droplets is passed along a tube which is usually heated.
- As the droplets proceed along the tube, solvent evaporates to yield smaller droplets.
- Because the electrically charged droplets retain their charge but get smaller, their electric field increases.
- At some point, mutual repulsion between like charges causes charged particles (*ions*) to leave the surface of the droplet (*ion evaporation*). These ions can be detected by the mass spectrometer.
- Additional ionization occurs by collision between the ions and other neutral species (ion/molecule collision - see 'Chemical Ionization'). Unless special steps are taken (See 'Thermospray', 'Plasmaspray', 'Electrospray' and 'MS-MS') the ions formed do not fragment so that little or no structural information is obtained. However, the lack of fragmentation does mean that good relative molecular mass data can be achieved. The assembly of ions formed by ion evaporation and chemical ionization passes through a small orifice (a *skimmer*) into the mass spectrometer for mass analysis in the usual way (see 'Ion Optics').
- Greater sensitivity is attained if the original solvent is polar (e.g. water or methanol).
- Greater sensitivity is attained if the solvent already contains ions through addition of an electrolyte.
- Greater sensitivity is attained if an additional ionization mode is included. This may take the form of a radioactive source, a heated filament or a plasma or glow discharge.
- All three of the last items can be combined for maximum sensitivity.

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- The rate of evaporation of solvent from droplets can be increased by 'blowing' a *drying gas* across the stream. Nitrogen is frequently used as the drying gas.
- Practical inlet systems for attaching an HPLC column to a mass spectrometer utilize atmospheric pressure ionization (see 'Thermospray', 'Plasmaspray', 'Electrospray')

Summary Evaporation of solvent from a spray of electrically charged droplets at atmospheric pressure eventually yields ions which can collide with neutral solvent molecules. The assemblage of ions formed by evaporation and collision is injected into the mass spectrometer for mass analysis.

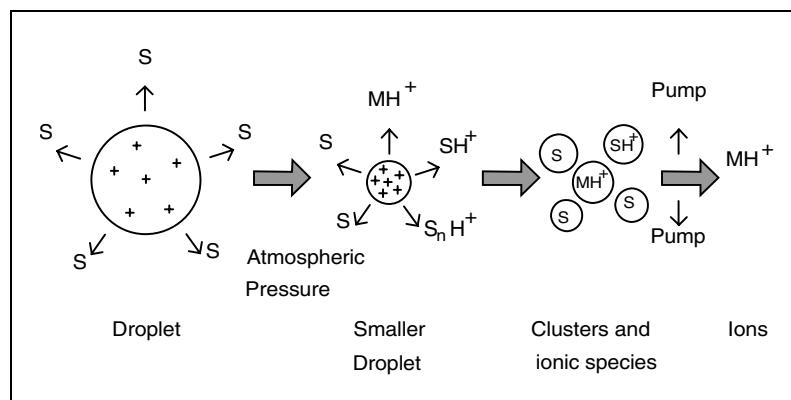


Figure I After being formed as a spray, many of the droplets contain some excess positive (or negative) electric charge. Solvent (S) evaporates from the droplets to form smaller ones, until eventually, ions (MH^+ , SH^+) from the sample M and solvent begin to evaporate to leave even smaller drops and clusters (S_nH^+ ; n=1, 2, 3 etc.). Later, collisions between ions and molecules (Cl) leave MH^+ ions which proceed on into the mass analyser. Negative ions are formed similarly.

ATMOSPHERIC PRESSURE IONIZATION

Background

Traditionally, electron and then also chemical ionization were the principal methods for producing ions for mass spectrometry. As these techniques need to operate in the gas phase, any sample of interest must be vaporized into the ion source. Whilst vaporisation of a sample is satisfactory for many substances, there are also large numbers of them which cannot be vaporized without thermal decomposition taking place. For example, sugars, peptides, proteins, nucleosides and so on fall into this category. In the same way, these sorts of substances will not pass through a gas chromatographic column. Although methods have been developed for derivatising involatile and thermally labile substances to make them volatile, these necessitate extra steps and, even so, some substances remain labile or involatile (e.g. proteins). High performance liquid chromatography as a complement to gas chromatography removed these analytical problems since no volatilisation was needed but the problem remained of getting these less volatile substances into a mass spectrometer. The advent of *atmospheric pressure ionization* (API) provided a method of ionizing labile and involatile substances so that they could be examined by mass spectrometry. API has become strongly linked to HPLC as a basis for ionizing the eluent on its way into the mass spectrometer, although it is also used as a stand-alone inlet for introduction of samples. API is important in thermospray, plasmaspray and electrospray, for which there are 'Back to Basics' issues.

The Ionization Process

(a) Ion Evaporation

When a stream of liquid (solvent) containing a sample (substrate) of interest is sprayed from the end of a narrow tube (nebulized) into a larger chamber, a mist of small droplets is produced which drift along the chamber (Figure 1).

A proportion of the droplets carry excess of positive or negative electric charge. The droplets have a large surface area-to-volume ratio and solvent evaporates quickly so that the droplets get smaller and the electric charge density increases. At some point, the mutual repulsion between like charges becomes so great that it exceeds the forces of surface tension and ions begin to leave the droplet as well as the neutral solvent molecules.

This process has been called *ion evaporation*. The ions may leave the droplets alone or associated with solvent molecules in clusters (Figure 1).

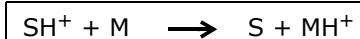


Figure 2 An example of proton (H^+) transfer from a protonated solvent molecule (SH^+) or cluster to form a quasimolecular ion (MH^+) of the substrate (M).

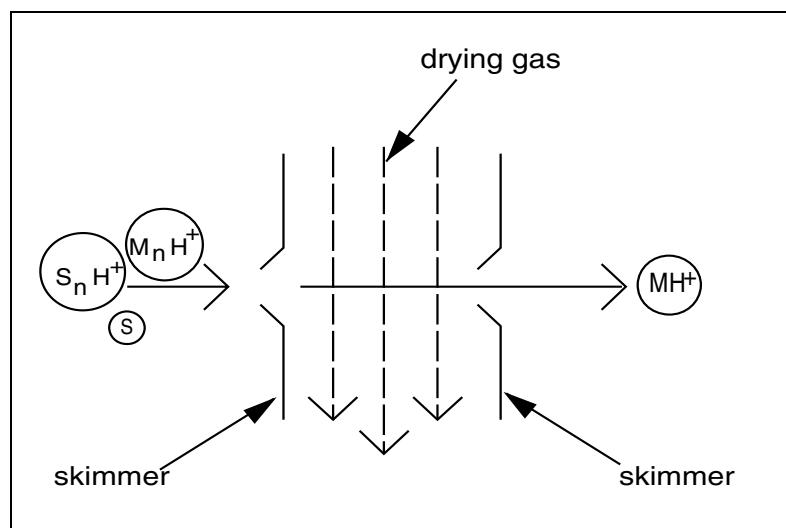


Figure 3 Use of a drying gas to remove more solvent (S) and reduce the number of cluster ions, (S_nH^+) or M_nH^+ or $\text{S}_m\text{M}_n\text{H}^+$; (m, n are integers).

As the mixture of ions and ion clusters approaches the mass spectrometer, further evaporation occurs and another mode of ionization becomes important.

(b) Chemical Ionization (CI)

Further explanation of this method can be found in 'Chemical Ionization' in this series. Briefly, CI works by collision between sample molecules and specially produced reagent gas ions such that ions are formed from sample molecules by various processes, one of the most important of which is the transfer of a proton (H^+ , Figure 2). Since ions and neutral molecules are formed close together in an API source, many ion/molecule collisions occur as in CI, and so the ion evaporation process also has impressed upon it the characteristics of CI. Therefore, API is usually thought to involve a mix of ion evaporation and chemical ionization.

The mix of ions, formed essentially at or near ambient temperatures, is passed through a nozzle (or skimmer) into the mass spectrometer for mass analysis. Since the ions are formed in the vapour phase without having undergone significant heating, many thermally labile and normally involatile substances can be examined in this way.

Drying Gas

To aid the evaporation of the droplets, a flow of a gas (often nitrogen) is directed across them. This drying gas helps to reduce the number of cluster ions (Figure 3).

The Number of Ions

Under the above conditions, the yield of ions is quite small because most droplets are either neutral or not highly charged electrically. Therefore, the sensitivity of mass spectrometry using simple API is low. Several means of improving the yield of ions have been developed:

- (a) The liquid (solvent) which is nebulized should be polar (e.g., water, acetic acid).
- (b) The liquid (solvent) should already contain ions by inclusion of an electrolyte.
- (c) Additional ionization is effected by including radioactive substances or plasma or glow discharges in the evaporation chamber or by electrical charging of the nebulizer. Such techniques are also discussed under the headings of Thermospray, Plasmaspray, APCI and Electrospray in 'Back to Basics'.

Conclusion

Evaporation from a spray of charged droplets produced from a stream of liquid gives ions which can be analysed in a mass spectrometer. Thermally labile and normally involatile substances such as sugars, peptides and proteins can be examined successfully.

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**Back to Basics Section B:
Interfaces and Ionization Techniques**

CHAPTER B3

Z-SPRAY COMBINED INLET/ION SOURCE

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Quick Guide

- Z-Spray is the name given to a novel kind of electrospray combined inlet and ion source. The Back-to-Basics Guide on Electrospray Ionisation should be consulted for comparison.
- For conventional electrospray, a solution of an analyte is sprayed from a narrow tube into a region, in which the solvent and other neutral molecules are pumped away and residual ions are directed into the analyser of a mass spectrometer.
- The spraying process is aided by placing a high electric potential on the end of the narrow tube, from which the solution exits and also by using a nebulising gas flowing past the end of the tube. Very small electrically-charged droplets are produced, positive for a positive electric potential and vice versa.
- Solvent evaporates from the droplets as they move towards an opening (skimmer) and then into the mass analyser.
- Before the skimmer is reached, much of the solvent has evaporated and mostly only residual ions carry on through the skimmer opening. The trajectory of the ions from the solution exit tube to the skimmer is a straight 'line-of-sight' in conventional electrospray sources.
- Before ions actually pass into the spectrometer analyser, there is usually a second 'drying' stage for removal of any final solvent.
- The ions, which pass into the analyser, have near ambient thermal energies and do not fragment but give excellent molecular or quasi-molecular ions. These ions may be investigated for their m/z values by almost any kind of mass analyser.
- Ions can be induced to fragment by increasing an electric potential known as a 'cone voltage', which speeds them up. Accelerating the ions causes them to collide more energetically with neutral molecules, a process that causes them to fragment (collision induced decomposition).
- The Z-spray source utilizes exactly these same principles, except that the trajectory taken by the ions before entering the analyser region is not a straight line but is approximately Z-shaped or, rather, a somewhat flattened Z-shape. Electrically neutral molecules are not deflected through this trajectory but flow towards the vacuum pumps.

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- The Z-trajectory ensures excellent separation of ions from neutral molecules at atmospheric pressure. In ‘line-of-sight’ or conventional electrospray sources, the skimmer is soon blocked by ions and molecules sticking around the edges of the orifice. In Z-spray sources, the final skimmer, being set off to one side, is not subjected to this build-up of material.
- Z-Spray sources require much less frequent maintenance than do conventional electrospray sources.

Summary

The Z-spray inlet/ion source is a particularly efficient adaptation of the normal ‘in-line’ electrospray source and gets its name from the approximate shape of the trajectory taken by the ions between their formation and their entrance into the analyser region of the mass spectrometer. A Z-spray source means that maintenance down-time for source cleaning is greatly reduced.

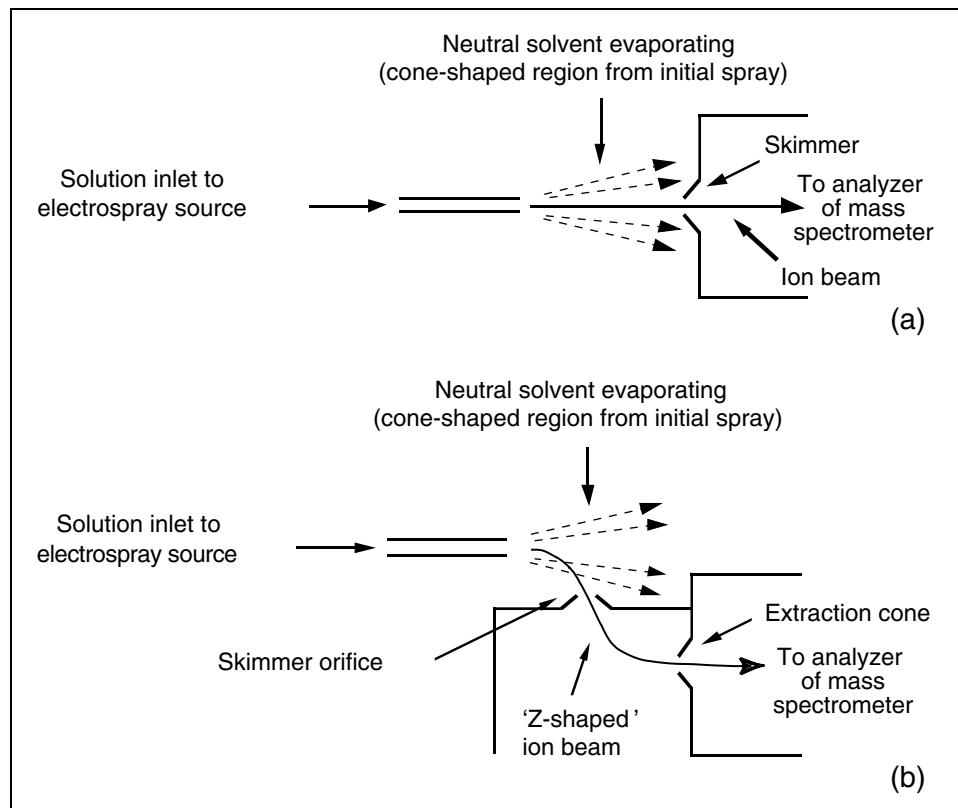


Figure I (a) The trajectory of analyte ions from a conventional electrospray inlet/ion source is essentially a straight line between the inlet tube carrying the solution of interest and a skimmer orifice placed a short distance away. The neutral solvent molecules are shown diffusing away from the main ion beam over a roughly cone-shaped space.
 (b) The trajectory of analyte ions from a Z-spray inlet/ion source follows a sort of flattened Z-shape in going from the inlet tube to the final skimmer. Again, neutral solvent molecules are shown diffusing away from the main ion beam over a roughly cone-shaped space. However, unlike the situation in (a), the ion beam first passes through an initial skimmer orifice placed such that it lies at right angles to the direction of the spray. Electrical potentials in the source cause the ion beam to bend towards the skimmer orifice and, having passed through it, to bend once again so as to pass through the extraction cone.

Z-SPRAY COMBINED INLET/ION SOURCE

Introduction

A solution of an analyte in a solvent can be sprayed (nebulized) from an electrically-charged narrow tube to give small electrically-charged droplets, which desorb solvent molecules to leave ions of the analyte. This atmospheric pressure ionisation is known in various forms, the one most relevant to this section being called electrospray. For greater detail, it is strongly recommended that the *Back-to-Basics Guide on Electrospray Ionisation* (and APCI, Thermospray and Plasmaspray) should be consulted.

As an adaptation of electrospray, Z-spray is cleaner and more efficient for the generation and separation of analyte ions from solvent and buffer agents. In conventional electrospray sources, droplets issue from the end of a narrow inlet tube as a cone-shaped spray, in which small molecular mass solvent molecules tend to diffuse away towards the edges of the cone and high molecular mass analyte ions continue along the axis of the cone until they enter the mass spectrometer analyser through a small orifice (the skimmer). Thus, the narrow solution inlet, the cone axis and the position of the orifice lie along one straight line-of-sight (Figure 1a). Approximately, ions produced in such a situation travel along a linear trajectory from formation to entering the analyser. Ions going through the skimmer are accompanied by small quantities of neutral materials and some of these as well as ions strike the edges of the skimmer and are deposited there. Gradually, the skimmer hole becomes blocked. The Z-spray inlet/ionisation source has a geometry such that the trajectory of the ions can be likened to a somewhat flattened Z-shape (Figure 1b) and hence the name. However, a final skimmer is set off to one side of the spray instead of being in-line. Any neutral species continue on to the vacuum pumps so that build-up of deposits and blockages are greatly reduced.

The initial spray

Two situations need to be considered depending on the type of inlet tube used. These last may be considered as 'normal' narrow tubes or very small diameter 'nanotubes'.

(i) Nanotube sprays

Nanotubes may be simply a short section of a capillary tube that holds a small quantity of the solution of interest (Figure 2a). Alternatively, Figure 2b shows this inlet as the exit from a liquid chromatography apparatus, which is equipped with very narrow 'nanocolumns'.

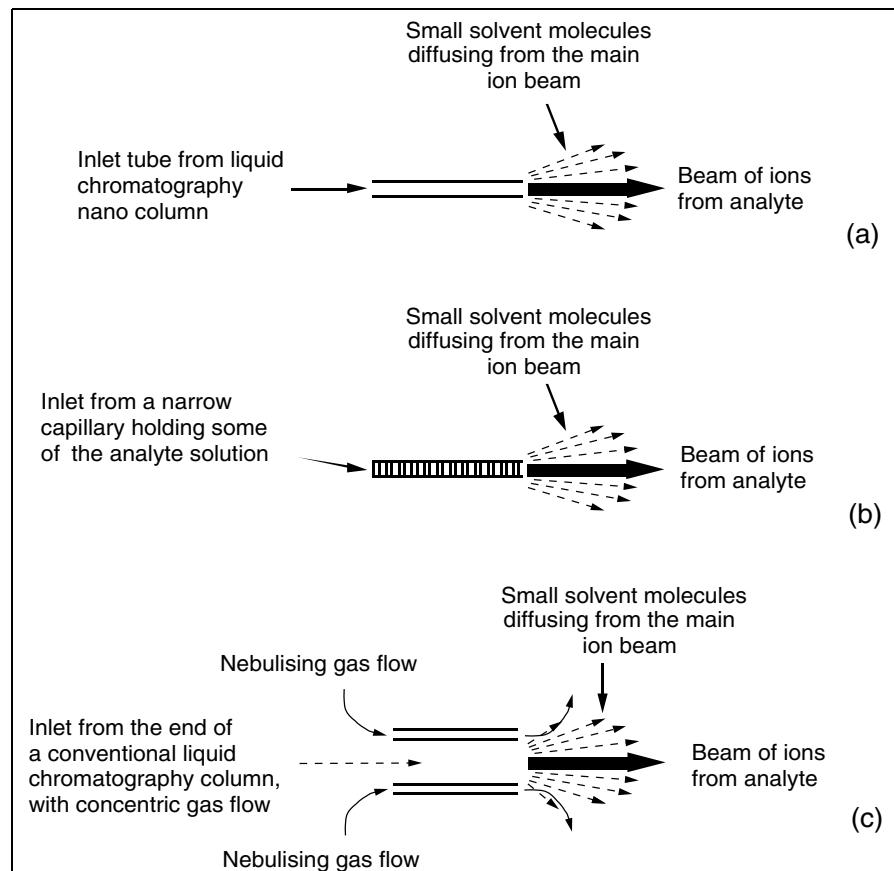


Figure 2 Initial trajectories for three kinds of inlet are illustrated. In (a), a high electrical potential of about 3 to 4 kV causes a spray of droplets to be ejected from the end of the inlet from a liquid chromatography nanocolumn as a solution flows to the end of the column. In (b), the same electrical potential applied to a stationary analyte solution held in a very narrow capillary again causes a spray of droplets from the end of the inlet tube, even though there is not the formal liquid flow existing in (a). As the electrically charged droplets move away from the end of the inlet tube, rapid evaporation of small neutral solvent molecules eventually leaves behind a beam of analyte ions. Although this ion beam is shown as tightly collimated, in fact it also gradually disperses but not as rapidly as for the solvent because of the higher molecular masses of analyte molecules compared with solvent molecules. In (c), the inlet from a wider 'normal' liquid chromatography column is shown. Now, to obtain a satisfactory spray, it is necessary to add a gas flow concentric with the inlet tube. This type of nebulisation is familiar from the common spray cans used, for example, for paint or hair lacquer (but without the high potential!!).

For the chromatographic column, flow of solution from the narrow inlet tube into the ionisation/desolvation region is measured in terms of only a few microlitres per minute. Under these circumstances, spraying becomes very easy simply by application of a high electrical potential of about 3 - 4 kV to the end of the nanotube. Similarly, spraying from any narrow capillary is also possible. The ions formed as part of the spraying process follow trajectories governed by electrical gradients in the inlet system; these are discussed below.

(ii) Normal inlet tube sprays

A common liquid chromatography column is somewhat larger in diameter than a nanocolumn. Consequently, the flow of solution along such a column is measured in terms of one or two millilitres per minute and spraying requires the aid of a gas flowing concentrically around the end of the inlet tube (Figure 2c). An electrical potential is still applied to the end of this tube to ensure adequate electrical charging of the droplets.

Trajectories of ions and neutrals

Once the spray has formed, electrically-charged droplets tend to carry on in a straight line according to their initial momenta gained in the electric field. However, neutral solvent molecules are not affected by the electric field and, as they evaporate from the charged droplets, they diffuse away, eventually to be drawn down into the pumping system. When much of the solvent has evaporated from the droplets, analyte ions also begin to desorb. Analyte ions and any neutral analyte molecules will normally have much greater molecular masses and momenta than the solvent molecules so that they tend not to diffuse away from the main axis of the spray so readily as the smaller solvent molecules. If there are any buffers in the original solution being sprayed, some of this is entrained with the analyte ions and molecules. After a short distance from the end of the inlet tube, the initial spray changes from being a mix of charged droplets to mainly a beam of analyte ions and neutrals, together with some buffer if present (Figure 3).

For conventional electrospray, there is a 'line-of-sight' from the end of the inlet tube to a small hole (the skimmer), through which many of the ions pass on their way to the mass analyser, accompanied with some neutral species. After the skimmer, there is a second stage of removal of residual solvent and neutrals before the ions proceed into the mass analyser proper (Figure 3).

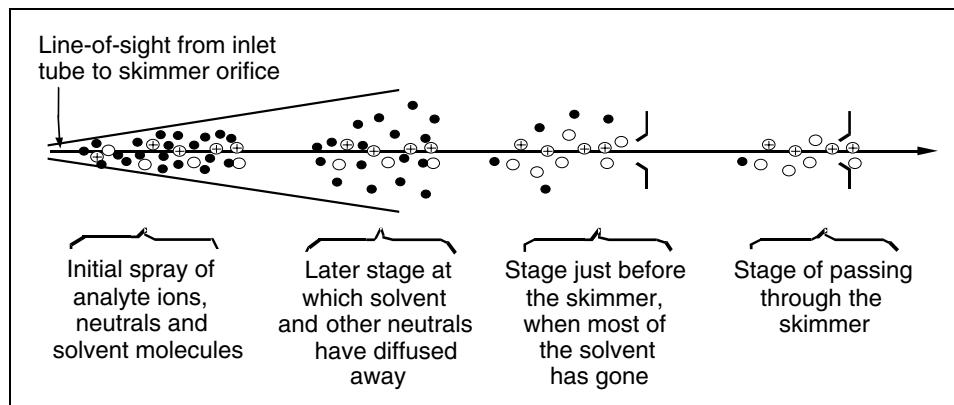


Figure 3 In a conventional electrospray inlet, the initial spray of electrically charged and neutral droplets forms a cone-shaped region. As low molecular mass solvent evaporates (small black circles), it diffuses away from the line-of-sight to the skimmer; neutral analyte molecules and analyte ions tend not to diffuse so quickly because of their generally much greater molecular mass and continue close to the line-of-sight. As the analyte ions and neutrals near the skimmer, there are few solvent molecules remaining so that, at the skimmer, mostly analyte ions and neutrals pass through. However, because of diffusion and mutual like-charged ion repulsion, the ion beam is not closely defined and some of it strikes the edges of the orifice, instead of passing through. This causes a build-up of material, which eventually blocks the orifice. The situation is exacerbated if the original solution flowing from the inlet tube contains non-volatile buffering agents, which cause much faster blocking of the skimmer and therefore more frequent cleaning.

The problem with this ‘linear’ arrangement lies in the species that do not quite make it through the skimmer hole. If these ions and neutrals strike the edges of the skimmer, some will stick there. Gradually, there is a build-up of unwanted material around the skimmer orifice, which is made smaller and smaller and can finally be blocked altogether. Clearly, as the hole diameter gets smaller, fewer and fewer ions will pass through it and the sensitivity of the instrument gets less and less. Even before the skimmer is totally blocked, a point is reached when there is no alternative to cleaning out the inlet to remove the built-up deposits before the instrument can be used again.

A Z-spray source gets round this problem by separating the trajectories of the neutrals and the ions since only the latter are affected by electric fields. Accordingly, a first skimmer orifice is moved from a ‘line-of-sight’ position to one at right angles to the initial spray direction (Figure 4). Now, as the ions form they are diverted by electric gradients in an arc away from the neutral molecules of solvent, analyte and any buffer, which continue straight on under their initial momenta or diffuse away and are pumped off. There is a drying gas flowing around the entrance to the skimmer to remove any small quantities of neutrals that might have diffused there (Figure 4). Thus, the species proceeding through the skimmer and into the ion block are almost entirely ions, which are finally turned through another inverse arc so as to pass through a second skimmer orifice (the extraction cone) into the mass analyser (Figure 4). The two opposed arcs of the ion trajectory form a sort of (flattened) Z-shape.

Advantages

With the Z-spray design, there is almost no build-up of products on the skimmer orifice so that instrumental sensitivity and performance stays constant over long periods of time. In addition, it is found that this arrangement is inherently a better ion collector than the line-of-sight mode, which gives a useful gain in instrumental sensitivity. The open arrangement resulting from the design gives better access to the inlet tube, allowing its easy manipulation. This is particularly important in the placing of nanotubes. Finally, collisionally activated decomposition (CAD) of ions can still be effected by an increase in the ion extraction voltage (cone voltage - see *Electrospray Ionisation*).

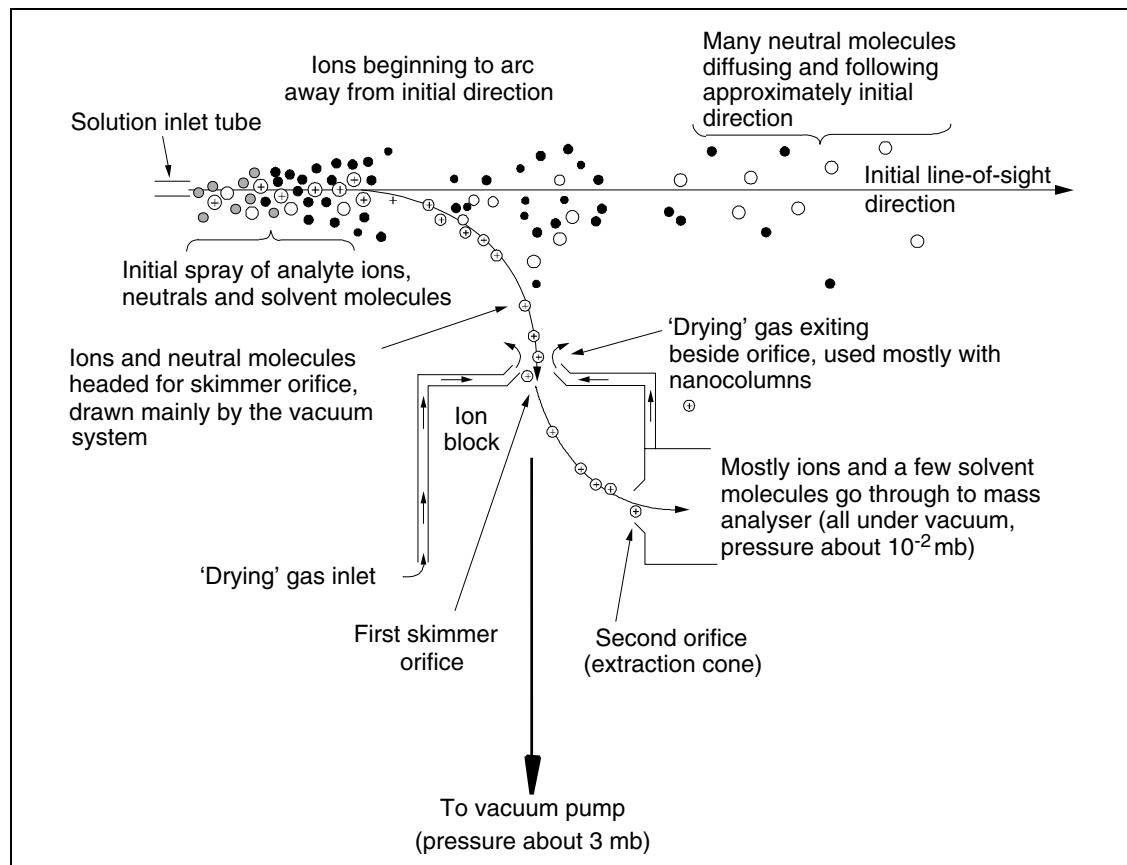


Figure 4 Solution issuing from the end of the inlet tube, held at an electrical potential of 3 - 4 kV, forms a spray of droplets at atmospheric pressure. Solvent evaporates from these droplets. Under the influence of the general gas flow towards the vacuum pumps, and partly due to the electric fields, ions and neutral molecules move in an arc through the first skimmer orifice, as shown. After this opening, a split between ions and neutral molecules is effected. Most remaining solvent and other neutrals flow on towards the first-stage vacuum pump. But an electric field gradient causes the ions to flow in an arc towards a second skimmer, often called the 'extraction cone', and on to the mass analyser. A few neutrals diffuse through this second skimmer, as well, because of the differences in pressures on either side of it. Note the overall flattened Z-shape of the ion trajectory.

Conclusion

By causing ions and neutrals to follow different paths after they have been formed from the electrically-charged spray produced from a narrow inlet tube, the ions can be drawn off into a mass analyser without any accompanying neutrals. The Z-shaped trajectory taken by the ions gives the inlet its name and ensures that there is little build-up of products on the narrow skimmer entrance into the mass spectrometer analyser region. Consequently, in contrast to a conventional electrospray source, the skimmer does not need to be cleaned frequently and the sensitivity and performance of the instrument remain constant for long periods of time.

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**Back to Basics Section B:
Interfaces and Ionization Techniques**

CHAPTER B4

**THERMOSPRAY AND PLASMASPRAY
INTERFACE**

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Quick Guide

- Thermospray is *both* a liquid *inlet* system for a mass spectrometer and, at the same time, an *ionization source*.
- Plasmaspray, or discharge-assisted thermospray, is a modification of thermospray in which the degree of ionization has been enhanced.
- As the name implies, thermospray uses heat to produce a spray of fine droplets. Plasmaspray does not produce the spray by using a plasma but, rather, the droplets are produced in a thermospray source and a plasma is added afterwards to increase the number of ions produced.
- In many applications of mass spectrometry it is necessary to obtain a mass spectrum from a sample dissolved in a solvent. The solution cannot be passed straight into the mass spectrometer because, in the high vacuum, the rapidly vaporizing solvent would entail a large pressure increase causing the instrument to shut down.
- Therefore, the sample solution, which may or may not come from an LC chromatographic column, is passed along a narrow capillary tube at the end of which it is strongly heated.
- The strong localized heating causes the liquid to vaporize very rapidly, forming a supersonic jet which leaves the end of the capillary as a mist of fine droplets mixed with vapour.
- The droplets, which are electrically charged, pass into and along a small vacuum chamber.
- As the droplets pass through the chamber, they evaporate and rapidly become much smaller through vaporization of solvent. At the same time, because the surface area of the droplets gets smaller and smaller, the density of electrical charge on the surface increases until a point of instability is reached.
- Eventually, not just neutral solvent molecules but also ions start to desorb from the surface. With most of the solvent removed and the pressure down to that of the mass spectrometer, these ions pass on into its analyser so that a mass spectrum can be obtained.

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- Unfortunately, although this is a simple system with no moving parts, not many ions from the original dissolved sample are produced and the thermospray inlet/ion source in itself is not very sensitive considering the achievable sensitivities of standard mass spectrometers.
- To increase the number of ions, a plasma discharge is produced in the mist issuing from the capillary. The electrical discharge induces more ionization in the neutrals which accompany the relatively few 'thermospray' ions. This enhancement means that more sample molecules are ionized and makes the technique much more sensitive; to distinguish it from simple thermospray, it is called plasmaspray.
- Ions formed by thermo- or plasmaspray are extracted through a small hole into the mass spectrometer analyser where a mass spectrum of the original dissolved sample is obtained.
- Thermospray and plasmaspray can be used with both sector and quadrupole instruments. They have been used extensively to couple liquid chromatographs to mass spectrometers.

Summary

A sample to be examined by thermospray is passed as a solution in a solvent (made up separately or issuing from a liquid chromatographic column) through a capillary tube which is heated strongly at its end so that the solution vaporizes and emerges as a spray or mist of droplets. As the droplets evaporate, residual ions are extracted into a mass spectrometer for analysis. Plasmaspray is a modification of thermospray designed to enhance the yield of ions.

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THERMOSPRAY AND PLASMASPRAY

Introduction

For a more detailed description of the ionization process inherent in thermospray and plasmaspray please consult 'Atmospheric Pressure Ionization' in these Back-to-Basics Guides. The reader should compare thermospray with electrospray (see relevant Guide).

In many applications in mass spectrometry the sample to be analysed is present as a solution in a solvent which could be, for example, methanol or acetonitrile or an aqueous one, as with body fluids. The solution may be an effluent from a liquid chromatography column. In any case, a solution flows into the 'front end' of a mass spectrometer but, before it can provide a mass spectrum, the bulk of the solvent must be removed without losing the sample (solute). If the solvent were not removed then its vaporization as it entered the ion source would produce a large increase in pressure and stop the spectrometer from working. At the same time that the solvent is removed, the dissolved sample must be retained so that its mass spectrum can be measured. There are several means of effecting this differentiation between carrier solvent and the solute of interest and thermospray is just one of them. Plasmaspray is a variant of thermospray in which the basic method for removal of solvent is the same but the number of ions obtained is enhanced (see below).

One of the first successful techniques for selectively removing solvent from a solution without losing the dissolved solute was to add the solution dropwise to a moving continuous belt. The drops of solution on the belt were heated sufficiently to evaporate the solvent and the residual solute on the belt was carried on into a normal EI or CI ion source where it was heated more strongly so that it in turn volatilized and could be ionized. The moving belt system had some mechanical problems and could be temperamental. It can still be found in some laboratories but the more recent, less mechanical inlets such as thermospray and electrospray are gradually displacing it.

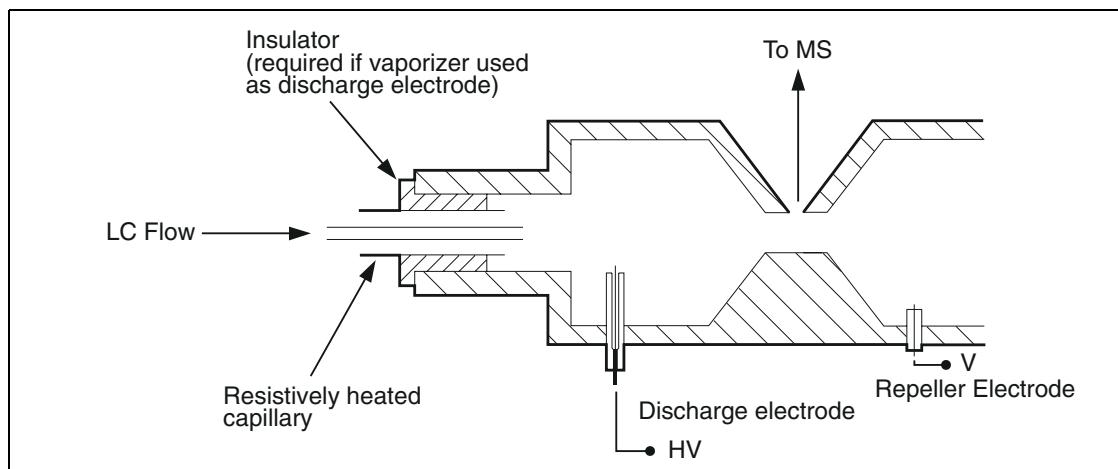


Figure I Schematic diagram of a thermospray ion source. This source, of current design, also incorporates a discharge electrode so that the source may be operated in plasmaspray mode and a repeller electrode so that fragmentation may be induced. The vaporizer may itself be used as a discharge electrode.

Differential Solvent Removal

A sample for which a mass spectrum is required may well be dissolved in an organic or an aqueous solvent. For example, in searching for drugs in blood plasma, the plasma itself may be investigated (aqueous) or its active components may be first extracted into an organic solvent such as dichloromethane. Alternatively, the sample may first be separated into its components by passage through a liquid chromatographic instrument (see Back-to-Basics Guide on LC); on emerging from the column the sample of interest is present as a solution in the solvents used in the chromatography. In either case, the sample to be examined is in solution and cannot be put straight into a mass spectrometer without first removing most of the solvent and without, of course, removing the dissolved sample also!

Thermospray is one method for effecting this differential solvent removal. The solution is passed along a short length of stainless steel capillary tube, the end of which is heated strongly by electrical resistive heating (Figure 1). When the solution reaches the hot zone, it is almost instantaneously vaporized and leaves the tube as a supersonic jet or spray of very small droplets of solution in solvent vapour. Before entering the mass spectrometer proper this mist of droplets flows along an evacuated tube which is continuously pumped down to a modest vacuum and the walls of which are heated slightly to prevent condensation. As the droplets move through this region, solvent evaporates rapidly from their surfaces and the droplets get smaller and smaller. In addition to producing the spray, this method of rapid vaporization leaves no time for equilibrium to be attained and a substantial proportion of the droplets have an excess of positive or negative electrical charge which resides on their surfaces. Thus, as the droplets get smaller the electrical surface charge density increases until such time that the natural repulsion between like charges causes ions as well as neutral molecules to be released from the surfaces (Figure 2). The end of the capillary tube is aimed somewhat like a gun at a solid (target) at the opposite end of this vacuum region. After vaporizing from the surface of a droplet, solvent molecules which have low molecular mass diffuse away from the 'line of fire' and are pumped off.

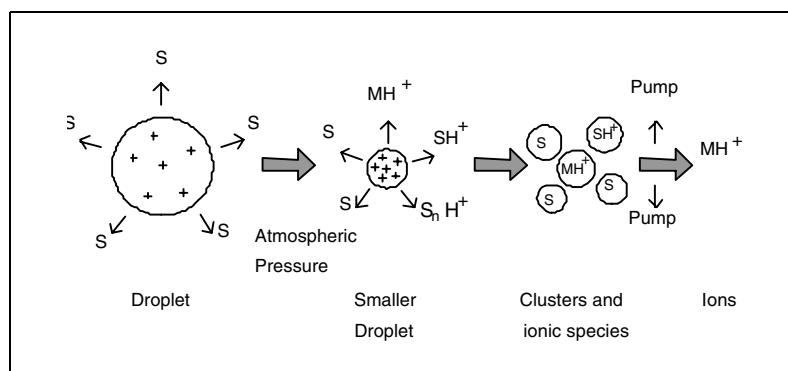


Figure 2 After being formed as a spray, many of the droplets contain some excess of positive (or negative) electric charge. Solvent (S) evaporates from the droplets to form smaller ones until, eventually, ions (MH^+ , SH^+) from the sample M and solvent begins to evaporate to leave even smaller drops and clusters ($S_n H^+$; n=1, 2, 3 etc.). Later, collisions between ions and molecules (Cl) leave MH^+ ions which proceed on into the mass analyser. Ion yield can be enhanced by including a volatile ionic compound (e.g., ammonium acetate) in the initial solution before it reaches the spraying zone.

However, the sample molecular ions and cluster ions have much greater molecular mass than those of the solvent and, being heavier, tend to carry straight on to the target at the end of the inlet vacuum region (Figure 1).

At the target, clusters are broken up and sample molecular ions, accompanied by some remaining solvent ions, are extracted by an electrical potential through a small hole into the mass spectrometer analyser (Figure 1) where their mass-to-charge (m/z) ratios are measured in the usual way. The mass spectrometer may be of any type.

The end result of the above process means that sample molecules dissolved in a solvent have been extracted from the solvent and turned into ions. Therefore, the system is both an inlet and an ion source and a separate ion source is not necessary.

Ion Yield

The thermospray inlet/ion source does not produce a good percentage yield of ions from the original sample, even with added salts (Figure 2). Often the original sample is present in very tiny amounts in the solution going into the thermospray and the poor ion yield leads to the thermospray/mass spectrometer becoming a relatively insensitive combination when compared with the sensitivity attainable by even quite a modest mass spectrometer alone. Various attempts have been made to increase the ion yield but, apart from one popular one, these are not described here. By placing a high electrical potential on an insulated electrode in the mist issuing from the heated end of the capillary tube, a plasma discharge can be struck. The spray of droplets passes through the negative (electrons) end of the plasma and electrons are stripped from neutral molecules such that the final yield of ions is greatly increased. Both solvent and solute (sample) ions are generated but, as before, mostly only the heavier sample ions are extracted into the analyser of the mass spectrometer. The addition of such a plasma device turns thermospray into plasmaspray, although it should be noted that the latter name is somewhat misleading in that the plasma does not cause the spray, which is still generated thermally.

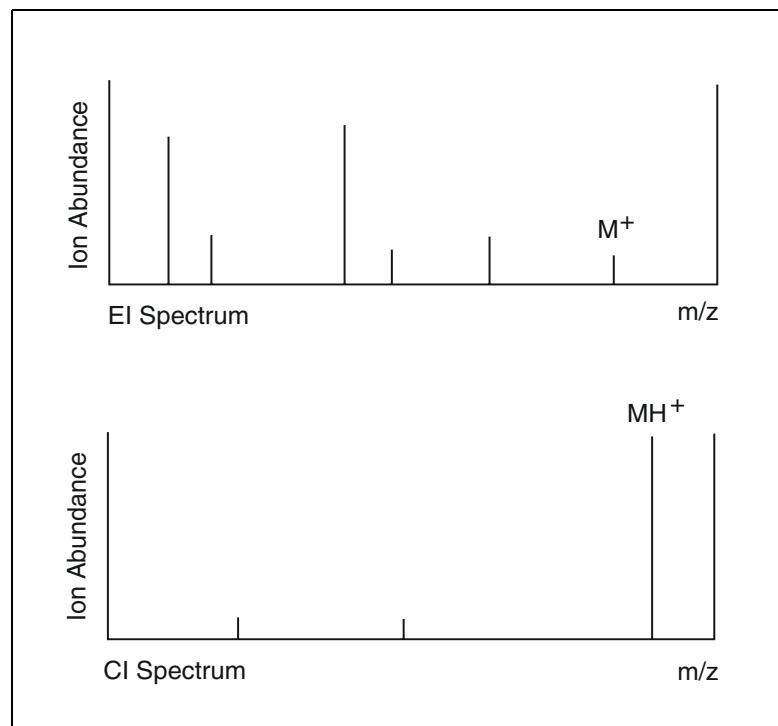


Figure 3 Comparison of EI and CI mass spectra illustrating the greater degree of fragmentation in the former and the greater abundance of quasimolecular ions in the latter.

Another way of improving ion yield is to include a repeller electrode (Figure 1). This electrode slows down lighter ions more than heavier ones which catch up and collide, causing enhanced chemical ionization.

Nature of the Ions Produced

Most of the ions produced by either thermospray or plasmaspray (with or without the repeller electrode) tend to be very similar to those formed by straightforward chemical ionization with lots of protonated or cationated positive ions or negative ions lacking a hydrogen (see Back-to-Basics Guide for CI). This is because, however formed, in the first part of the inlet the ions continually collide with neutral molecules in the early part of their transit, where the vacuum is not very high. During these collisions, the ions lose excess of internal energy, interchange protons and lose hydrogen atoms. These processes are predominant ones in chemical ionization. Plasmaspray mass spectra show some fragment ions and the repeller electrode leads to more but the degree of fragmentation is not normally comparable to that found in electron ionization spectra.

The ions passing into the mass spectrometer analyser from thermospray or plasmaspray have little excess of internal energy and therefore not enough energy to fragment. Most of the ions are of the form $(M+X)^+$ or $(M-H)^-$ in which X may be hydrogen or some other element such as sodium or potassium. Whilst these ‘quasimolecular’ ions are excellent for giving accurate molecular mass information, and this may be all that is required, they give little or none of the information concerning the actual molecular structure of the substance being investigated which is provided by fragment ions. Compare the typical EI and CI mass spectra illustrated in Figure 3. This is entirely analogous to the problem with simple chemical ionization and similar solutions to it exist. To give the quasimolecular ions the extra energy needed for them to fragment, they may be passed through a ‘collision gas’ and the resulting spectra analysed for metastable ions or by MS/MS methods (*loc. cit.*).

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Uses of Thermospray and Electrospray

Although simple solutions can be examined by these techniques, it is usually the case that, for a single substance dissolved in a solvent, straightforward evaporation of the solvent outside the mass spectrometer with separate insertion of the sample is sufficient. For mixtures the picture is quite different. Unless the mixture is to be examined by MS/MS methods, it will be necessary to separate it into its individual components. This is most often done by gas or liquid chromatography.

In the latter, small quantities of emerging mixture components dissolved in elution solvent would be laborious to deal with if each component were to be first isolated by evaporation of solvent before its introduction into the mass spectrometer. In such circumstances, the direct introduction, removal of solvent and ionization provided by thermospray or electrospray is a boon and puts LC/MS on a par with GC/MS for mixture analysis. Further, GC is normally concerned with volatile, relatively small molecular weight compounds and is of little or no use for the many polar, water soluble, high molecular mass substances such as the peptides, proteins, carbohydrates, nucleotides and similar found in biological systems. In contrast, LC/MS with thermospray or plasmaspray interface is used frequently in biochemical type research and medicine.

General Comments

Thermospray alone is not a very sensitive technique and is mostly, even then, of use only for polar compounds. It is now rarely used on its own because its plasma discharge assisted variant, plasmaspray, gives better results and ion yields and it faces completion from electrospray. Further, thermospray alone needs a solution containing electrolytes (ionic substances) if ions are to be formed. Since these electrolytes prevent reversed phase liquid chromatography from being used and often lead to blocking of the capillary inlet, there are added cogent reasons for moving to plasmaspray or electrospray.

Conclusion

By rapidly vaporizing a solution by heat, a spray is produced from which the solvent can be removed, leaving sample ions to pass straight into the analyser region of a mass spectrometer. Plasmaspray is very similar. The two inlet/ion sources are used most frequently as LC/MS interfaces.

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CHAPTER B5

PARTICLE - BEAM INTERFACE (LINCTM)

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Quick Guide

- The LINCTM interface is used to remove solvent from a liquid stream without, at the same time, removing the solute (or substrate).
- A flow of liquid, for example from HPLC, is treated in such a way that most of the solvent evaporates to leave solute molecules which pass into an ionization region (ion source).
- A stream of liquid issuing from a narrow tube can be broken up into a spray of small droplets by injecting helium gas just before the end of the tube. This *nebulisation* is analogous to the action of an aerosol spray-can nozzle.
- The flow of droplets enters an evaporation chamber which is heated sufficiently as to prevent condensation.
- Solvent evaporates from the droplets.
- The mix of tiny drops is formed into a ‘*particle beam*’ on passing through the exit nozzle of the evaporation chamber.
- The beam of tiny drops passes from the exit nozzle across an evacuated space and into another small orifice (‘skimmer 1’). In this evacuated region, about 90% of the originally injected helium and solvent is removed by vacuum pumps to leave a stream of droplets, so small that they are called *clusters*.
- The clusters are composed of aggregates of solvent (S_m), substrate (M_n) and mixed substrate/solvent molecules ($S_m.M_n$), where m, n are integers (1,2,3 etc.).
- The particle stream then passes through a second evacuated region between skimmer 1 and a second orifice (skimmer 2), when more residual solvent and helium are removed.
- Finally, the beam, mainly composed of single substrate and solvent molecules and very small clusters is passed through a heated wire grid where the last declustering and desolvation occurs to leave a beam of substrate molecules.
- The beam of substrate molecules then passes straight into the ion source (EI or CI) for ionization before entry into the mass analyser.

Summary

A stream of a liquid solution can be broken up into a spray of fine drops from which, under the action of aligned nozzles (skimmers) and vacuum regions, the solvent is removed to leave a beam of solute molecules, ready for ionization. The collimation of the initial spray into a linearly directed assembly of first droplets, then clusters and then single molecules gives rise to the term ‘Particle Beam Interface’.

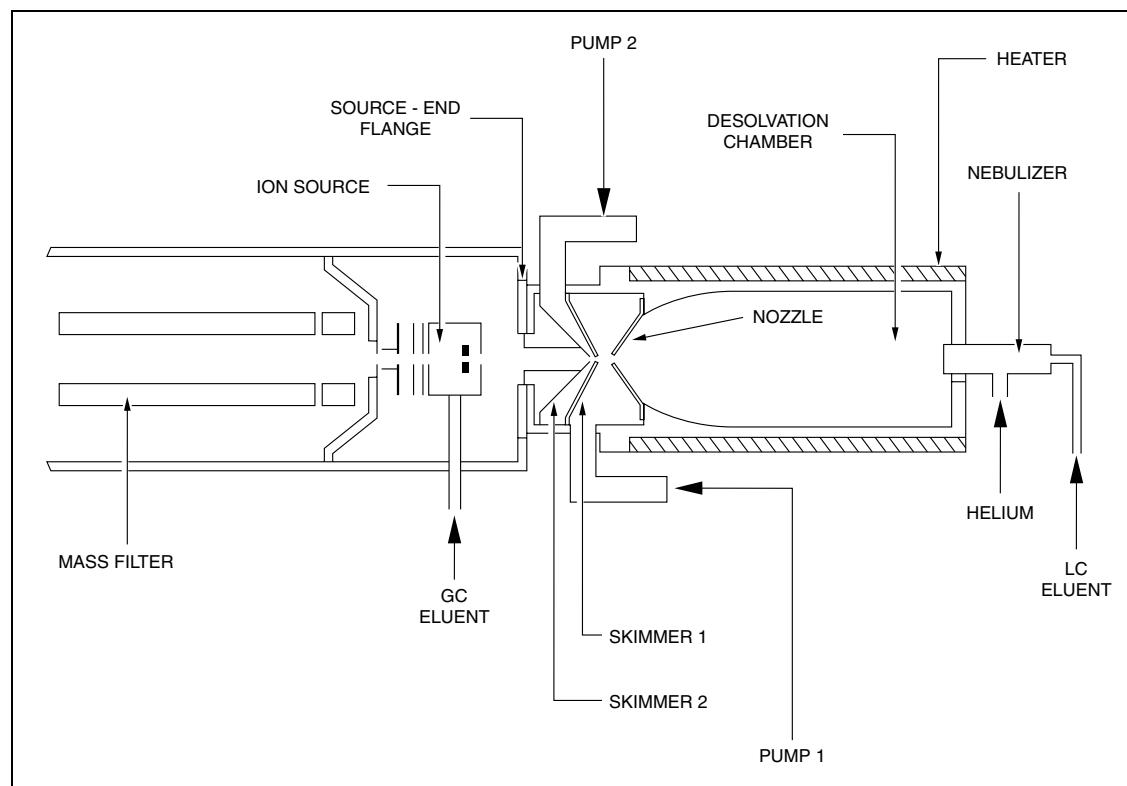


Figure 1 A typical arrangement for producing a particle beam from a stream of liquid, showing: (1) the nebulizer (2) the desolvation chamber, (3) the wall heater, (4) the exit nozzle, (5, 6) skimmers 1, 2, (7) the end of the ion source, (8) the ion source and (9) the mass analyser. An optional GC inlet into the ion source is shown.

PARTICLE BEAM INTERFACE (LINCTM)

- Background** The technique of GC/MS, whereby the gaseous effluent from a gas chromatograph can be passed into a mass spectrometer, has proved to be an enormously successful analytical method for volatile substances. Similar development for HPLC/MS or LC/MS as an analytical method for less volatile or polar materials was severely hindered by the need to remove the liquid solvent from the HPLC eluant. During passage from a liquid eluant at atmospheric pressure to a vaporized eluant needed for injection into the mass spectrometer, so much solvent vapour is formed that the spectrometer vacuum system is overwhelmed. A number of attempts has been made to separate solvent from solute without losing much of the solute (substrate). The particle beam interface (LINCTM) is one such successful method.
- The Nebulizer** As a first stage, the stream of liquid from an HPLC eluant is passed through a narrow tube toward the LINC interface. Near the end of the tube, the liquid stream is injected with helium gas so that it leaves the end of the tube as a high velocity spray of small drops of liquid mixed with helium and enters an evacuation chamber (Figure 1). The formation of spray (nebulizing) is very similar to that occurring in the action of aerosol spray-cans.
- The Evacuation Chamber** The small drops from the Nebulizer have a large surface area-to-volume ratio and solvent begins to evaporate rapidly from their surfaces; generally, solute or substrate is much less volatile than solvent and starts to concentrate as the drops get smaller (Figure 2). The chamber is heated to prevent evaporating solvent from condensing on its inner walls. The spray of drops finally leaves the evacuation chamber as a fast moving, slightly spreading beam – the beginning of the ‘particle beam’.

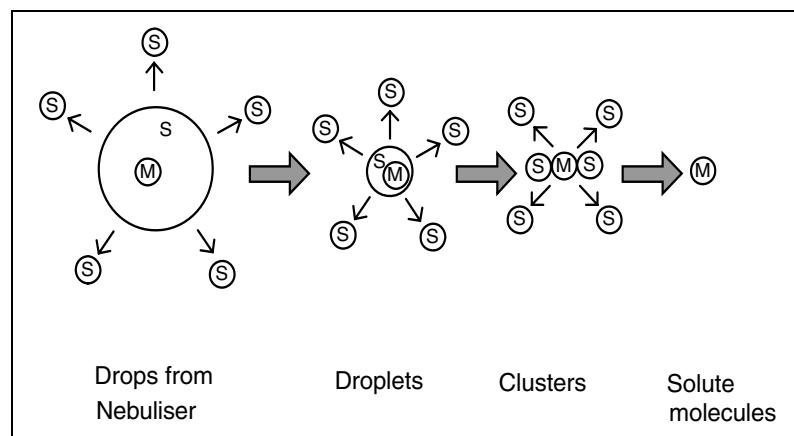


Figure 2 The passage of drops of solvent (S) containing a solute (M) through the evacuation chamber and the exit nozzle and skimmers 1, 2 into the ion chamber. Molecules of solvent evaporate throughout this passage causing the drops to get smaller until only solute molecules remain.

The First Skimmer	The flow of droplets is directed through a small orifice (Skimmer 1; Figure 1) and across a small region which is kept under vacuum by rotary pumps. In this region, approximately 90% of solvent and injected helium is removed from the incipient particle beam. Because the rate of diffusion of a substance is inversely proportional to its molecular mass, the lighter helium and solvent molecules diffuse away from the beam and are pumped away. The heavier solute molecules diffuse more slowly and pass through the first skimmer before they have had time to leave the beam; the solute is accompanied by residual solvent and helium.
The Second Skimmer	The beam from the first skimmer is directed toward a second one (Figure 1), again across an evacuated region where almost all of the residual solvent and helium are pumped away to leave a beam now consisting mostly of heavier solute molecules, together with small clusters of solvent and solute molecules. The gas pressure after this region has then been reduced from atmospheric to about 10^{-4} mb.
The Ion Source	The particle beam, after linear passage from the evacuation chamber nozzle, through the first and second skimmers and into the end of the ion source, finally passes through a heated grid, immediately before ionization. The heated grid has the effect of breaking up most of the residual small clusters so that residual solvent evaporates and a beam of solute molecules enters the ionization chamber.
Ionization	The beam entering the ion chamber is suitable for both electron (EI) and chemical (CI) ionization and both modes can be used (Figure 3). Mass analysis follows in the usual way, typically using quadruple or magnetic sector instruments.

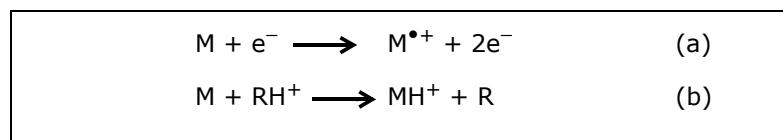


Figure 3 The ionization of solute molecules (M) by (a) EI, and (b) CI. A typical reagent gas is shown as RH^+ .

Efficiency	The efficiency of separation of solvent from solute varies with their nature and the rate of flow of liquid from the HPLC into the interface. Volatile solvents like hexane can be evaporated quickly and tend not to form large clusters and therefore rates of flow of about 1ml/min can be accepted from the HPLC apparatus. For less volatile solvents like water, evaporation is slower, clusters are less easily broken down and maximum flow rates are about 0.1 - 0.5 ml/min. Because separation of solvent from solute depends on relative volatilities and rates of diffusion, the greater the molecular mass difference between them, the better the efficiency of separation. Generally, HPLC is used for substances which are relatively involatile or are thermally labile, as these would otherwise be analysed by the practically simpler GC method; the involatile substances usually have molecular masses considerably larger than those of commonly used HPLC solvents so that separation is good.
Similarity to Other Interfaces	The nebulisation and evaporation processes used for the particle beam interface have closely similar parallels with Atmospheric Pressure Ionization (API), Thermospray (TS), Plasmaspray (PS) and Electrospray (ES) combined inlet/ionization systems (see 'Back to Basics' guides). In all of these, a stream of liquid, usually but not necessarily from an HPLC column, is first nebulized and the solvent is then selectively removed from the solute. However, LINC TM aims to provide a particle beam which is <i>ready for ionization</i> (by EI or CI) whilst the other methods provide a particle beam which is <i>already ionized</i> . Thus, the particle beam (LINC TM) interface is strictly an inlet system but API, TS, PS and ES provide both inlet and ionization combined. The major difference between these last and LINC TM lies in the additional measures taken to ensure ionization in the inlet so that no separate ion source is needed. From a practical viewpoint, techniques such as electrospray tend to give only quasimolecular and no fragment ions so that an additional means must be provided to cause fragmentation. The LINC TM method, with its separate ion source, provides molecular, quasimolecular and fragment ions by using a conventional EI and/or CI source.
Conclusion	The particle beam interface (LINC TM) works by separating unwanted solvent molecules from wanted solute molecules in a liquid stream that has been broken down into droplets. Differential evaporation of solvent leaves a beam of solute molecules which is directed into an ion source.

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CHAPTER B6
DYNAMIC FAB LSIMS INTERFACE

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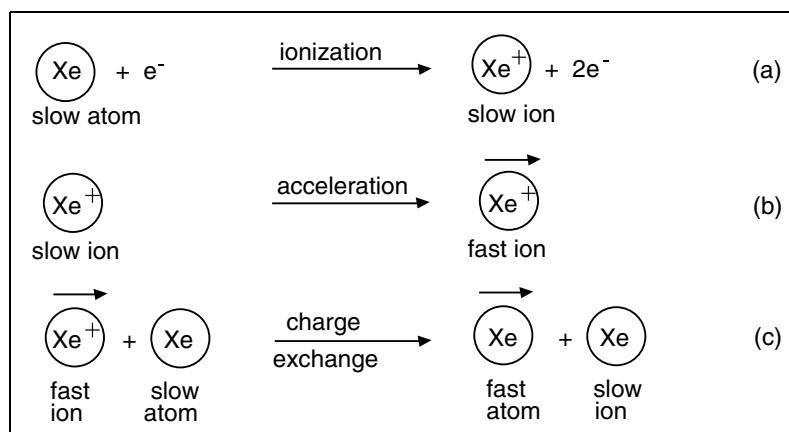
Quick Guide

- In fast atom bombardment (FAB), an atom gun is used to fire 'heavy' fast atoms at the static surface of a target solution (also called a matrix).
- In dynamic FAB, this solution is not stationary but flows steadily over the target area. Usually, the liquid flow is the eluant from a liquid chromatography column but need not be.
- The solution or matrix consists of the substance under investigation (the solute) dissolved in a solvent of high boiling point which evaporates only slowly in the vacuum of the mass spectrometer at an operating temperature of about 20-30°C.
- On leaving the chromatographic column, the liquid flow passes along a narrow tube and into the FAB ion source and thence into the target zone of the fast atoms.
- The impact of fast atoms on the solution surface results in desorption of secondaries (positive ions, negative ions and neutrals) into the low pressure gas phase above the target matrix surface.
- By selecting either a large positive or negative electrical potential on a plate with a slit in it held above the target area, the desorbed negative or positive ions respectively are extracted into the analyser of the mass spectrometer.
- The spectrometer provides a mass spectrum of the ions, some of which come from anything dissolved in the solution or matrix (solute ions) and some from the matrix solvent itself.
- Components of a mixture emerging from a liquid chromatographic column are dissolved in the eluting solvent and this solution is the one directed across the target, as described above. Thus, as the components reach the target they produce ions. These ions are recorded by the spectrometer as an ion current.
- The passage of a component of a mixture over the atom gun target area is accompanied by first a rise and then a fall in the ion current and a graph of ion yield against time is an approximately triangular-shaped peak.
- A graph or chart of ion current (y axis) versus time (x axis) is therefore a succession of peaks corresponding to components eluting from the chromatographic column. This chart is called a Total Ion Current chromatogram (TIC).

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- The area under each peak represents the amount of substance eluting from the column and the time at which it emerges is called the retention time for that component.
- Dynamic FAB is an interface between a liquid chromatograph and a mass spectrometer and is also, at the same time, an ion source. As an inlet/ion source, this technique fulfils a similar function to plasmaspray and electrospray both of which are also combined inlet/ion sources.
- Instead of bombarding the matrix with fast atoms, fast ions (FIB) can be used. Often these are caesium ions (Cs^+). As with fast atoms, fast ions cause desorption of ions and neutrals from the surface of a bombarded matrix.
- The term Liquid Secondary Ion Mass Spectrometry (LSIMS) is sometimes used synonymously with FAB and is preferred by some as being more descriptive since FAB could apply to bombardment of solid or liquid surfaces and does not indicate the types of secondaries being investigated. In practise, little confusion is likely to result from using either term. Strictly, LSIMS should refer to the use of fast ions (FIB), rather than fast atoms (FAB).

Summary By allowing any solution, but particularly the eluant from a liquid chromatographic column, to flow continuously (dynamically) across a target area under bombardment from fast atoms or ions (FAB or FIB), any eluted components of a mixture are ionized, the resulting ions being detected and recorded by a mass spectrometer. The technique is called dynamic FAB or dynamic LSIMS.

**Figure I**

- (a) Xenon atoms are ionized to Xe^+ , using electrons. These ions are relatively slow and move in all directions.
- (b) Xe^+ ions are accelerated through a large electric potential so that they attain a high speed in one direction.
- (c) Charge exchange between fast Xe^+ ions and slow Xe atoms gives a beam of fast Xe atoms and slow ions. The latter are removed by electric deflector plates, leaving just a beam of fast atoms.

Introduction

The basic principles of FAB (LSIMS) are discussed only briefly here because a fuller description appears in the 'Back-to-Basics' guide entitled 'FAB and LSIMS Ionization'. In this guide, the use of FAB/LSIMS is focused on its role as part of an interface between a liquid chromatograph and a mass spectrometer, although some theory is presented.

Bombardment of a liquid surface by a beam of fast atoms or of fast ions causes continuous desorption of ions which are characteristic of the liquid. Where the liquid is a *solution* of a sample substance dissolved in a solvent of low volatility (often referred to as a matrix), both positive and negative ions characteristic of the solvent and the sample itself leave the surface. The choice of whether to examine the positive or the negative ions is effected simply by the sign of an electrical potential applied to an extraction plate held above the surface being bombarded. Usually, few fragment ions are observed and a sample of mass M in a solvent of mass S will give mostly $[M+H]^+$ (or $[M-H]^-$) and $[S+H]^+$ (or $[S-H]^-$) ions. Therefore, the technique is particularly good for measurement of relative molecular mass.

The FAB source operates near room temperature and ions of the substance of interest are 'lifted' out from the matrix by a momentum transfer process which deposits little excess of vibrational and rotational energy in the resulting quasimolecular ion. Thus, a further advantage of FAB (LSIMS) over many other methods of ionization lies in its gentle or mild treatment of thermally labile substances such as peptides, proteins, nucleosides, sugars and so on which can be ionized without degrading their structures.

Liquid chromatography is a separation method which is often applied to involatile, thermally labile materials such as peptides and if their mass spectra are required after the separation step then a mild method of ionization is needed. Since FAB (LSIMS) is mild and works with a liquid matrix, it is not surprising that attempts were made to utilize this ionization source as both an inlet and an ion source capable of linking a liquid chromatograph directly to a mass spectrometer. Dynamic FAB is the adaptation of FAB (LSIMS) to forming an interface between a liquid chromatographic and a mass spectrometric instrument.

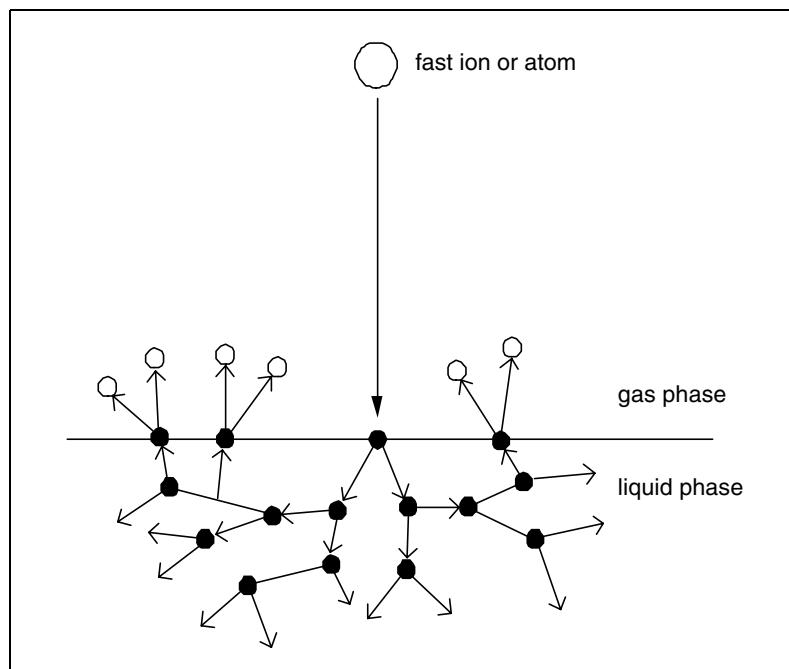


Figure 2 A typical cascade process. A fast atom or ion collides with surface molecules, sharing its momentum and causing the struck molecules to move faster. The resulting fast moving particles then strike others, setting up a cascade of collisions until all the initial momentum has been dissipated. The dots (●) indicate collision points. Ions or atoms (○) leave the surface.

Atom or Ion Beams

A ‘gun’ is used to direct a beam of fast atoms (often Xe) or fast ions (often Cs⁺) onto a small metal target area where the solution of interest is placed. Production of an atom beam is described in Figure 1. In dynamic FAB this solution is the eluant flowing from an LC column, i.e., the target area is covered by a flowing liquid (dynamic) rather than a static one as is usually the case where FAB is used to examine single substances. The fast atoms or ions from the gun carry considerable momentum and when they crash into the surface of the liquid, some of this momentum is transferred to molecules in the liquid which ‘splash’ back out, rather like throwing a stone into a pond (Figure 2). This is a very simplistic view of a complex process which also turns the ejected particles into ions (see Guide on FAB/LSIMS Ionization).

Properties of the Solvent (Matrix Material)

Liquids examined by FAB are introduced into the mass spectrometer on the end of a probe inserted through a vacuum lock in such a way that the liquid lies in the target area of the fast atom or ion beam. In this region, there is a high vacuum and there would be little point in attempting to examine a solution of a sample in one of the commoner volatile solvents such as water or dichloromethane because it would evaporate extremely quickly, probably as a burst of vapour on introduction into the vacuum. Therefore it is necessary to use a high boiling solvent as the matrix material, such as one of those listed in Table 1 at the end of this guide.

The solvents used for liquid chromatography are the commoner ones such as water, acetonitrile and methanol and, for the reasons just stated, it is not possible to put them straight into the ion source without problems arising. On the other hand, the very viscous solvents which qualify as matrix material are of no use in liquid chromatography. Before the low boiling point eluant from the LC column is introduced into the ion source it must be admixed with a high boiling point matrix material. Generally, addition of some 10% of matrix material (e.g., glycerol; see Table 1 at the end of this guide) is sufficient. It may be added with the solvents entering the LC column, or it may be admixed with the eluant leaving the column. The latter mode is better for LC separations.

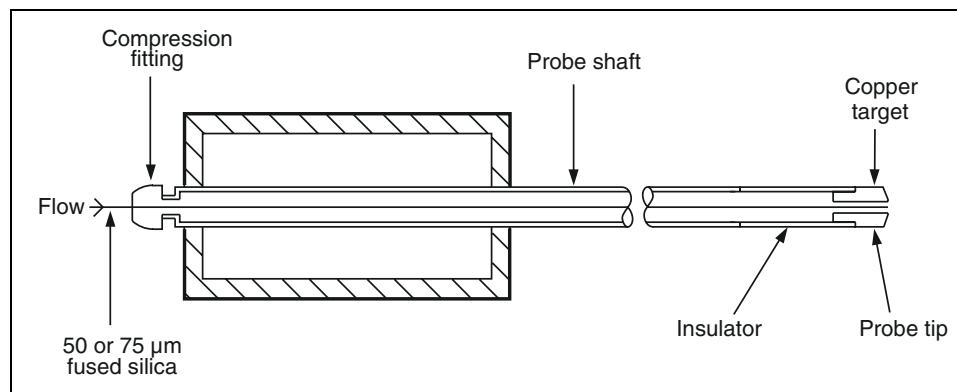


Figure 3 A dynamic FAB probe having a simple copper target. The narrow fused silica tube passes through the shaft, its end lying flush with the target surface.

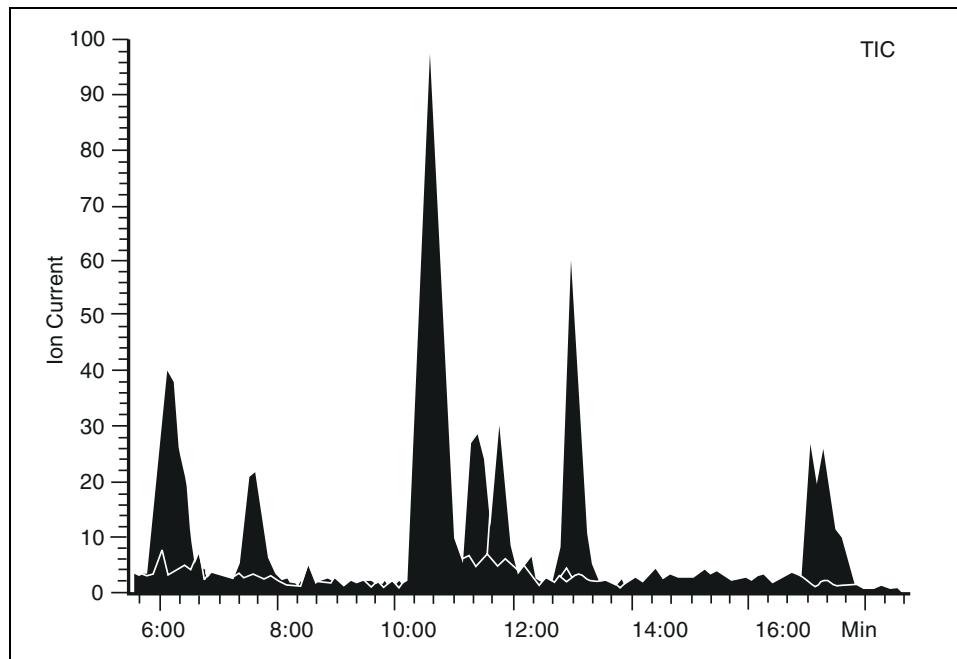


Figure 4 A typical total ion current chromatogram (TIC) from an analysis of peptides resulting from enzymic digest of myoglobin. The peaks represent individual peptides eluting from an LC column and being mass measured by a spectrometer coupled to it through a dynamic FAB inlet/ion source.

Having considered the various parts of a dynamic FAB system (atom gun, ionization and matrix), it is now necessary to see how these are put together in a working inlet/ion source interface.

Dynamic FAB/LSIMS Interface

One of the earliest models is illustrated in Figure 3 as it shows clearly the principles used in later improvements. The LC effluent was pumped along a length of silica capillary tubing inside a protective metal sheath (the probe shaft; Figure 3) which passed through a vacuum lock. At the end of the capillary the effluent reached a small copper target at the probe tip and flowed out from there. If flow rates are not too high (5 ml/min.) so that just enough liquid reaches the tip to balance that evaporating in the vacuum then the system is reasonably stable and fresh solution is continuously presented to the atom or ion beam. With only eluting solvent flowing the resulting mass spectrum consists of ions arising only from the solvent and added matrix. These ions are of relatively small mass and, by operating the mass spectral scan only above about m/z 200, few extraneous ions are recorded. When a component from a mixture which has been separated on the LC column reaches the target area it too is ionized and affords a mass spectrum. Thus, as components elute from the column and reach the target area, the recorded ion current rises from its background level and then falls again as the component passes beyond the target area. The ion current traces out a roughly triangular-shaped peak with time. A chart plotting the ion current (y axis) versus time (x axis) is then a *Total Ion Current* (TIC) chromatogram, an example of which is shown in Figure 4. The area under a peak is a measure of the amount of eluting component and the elapsed time from the start of the chromatogram to the maximum of the peak is its retention time.

More recent versions of this type of probe include some refinements such as the provision of a 'wick' to aid evaporation of the solvent and matrix from the probe tip (Figure 5). Such improvements have allowed greater flow rates to be used and rates of 1-10 ml/min are possible. For these sorts of low flow rates minibore LC columns must be employed.

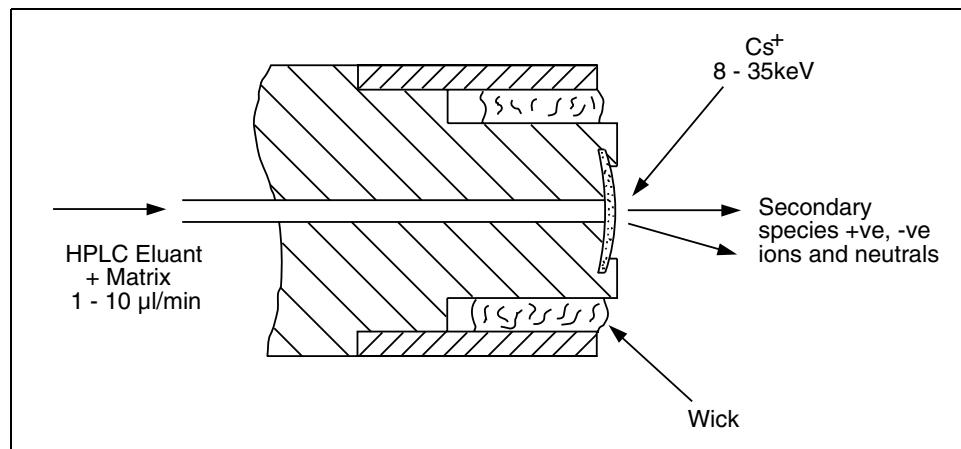


Figure 5 A dynamic-FAB probe tip incorporating a screen and wick assembly at the target surface.

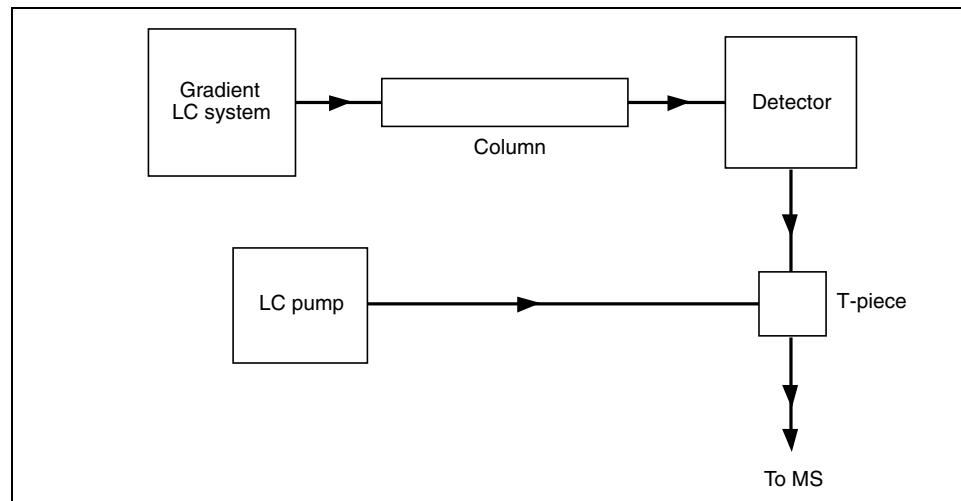


Figure 6 HPLC system incorporating a post-column addition facility. Note the pump used for post column addition must be essentially pulse free.

As mentioned above, matrix material must be added to the normal eluting solvent used in the LC system before the solvent stream enters the FAB inlet. The simplest way of effecting this addition is to premix about 10% of the matrix material with the solvent before it enters the LC column; this is done by dissolving about 10% of matrix in the elution solvent. Although very convenient, this pre-addition often leads to undesirable changes in the effectiveness of the column in separating components of a mixture. For example, introduction of the commonly used glycerol as matrix material can lead to serious 'tailing' (distortion of the peak shape) of the mixture components on the LC column. The best compromise is to add the matrix material to the solvent as it elutes from the column and before it enters the inlet of the dynamic FAB probe. Figure 6 presents one simple way in which this post-column addition can be done through use of a second pump. The total set-up may take about 30 minutes to stabilize before it can be used for analysis and is checked by watching for the ion source pressure reading to become constant.

Types of Ions Produced

Mostly, positive ion FAB yields protonated quasimolecular ions, $[M+H]^+$ and the negative ion mode yields $[M-H]^-$. In the presence of metal salts (e.g., KCl) which may be added to improve efficiency in the LC column, ions of the type $[M+X]^+$ are common, where X is the metal. Another type of ion which is observed is the so-called 'cluster', a complex of several molecules with one proton, $[M_n+H]^+$ with n = 1, 2, 3... etc. Few fragment ions are produced.

In static FAB (LSIMS) small abundances of ions appear at almost every m/z value and are colloquially referred to as 'grass'; these ions represent general degradation of the liquid being bombarded by the fast atom or ion beam. If the ion corresponding to the molecular mass of the sample under investigation has only low abundance, it may be difficult to differentiate it from the grass. This effect is particularly serious at high mass. In dynamic FAB, these degradation products do not have time to accumulate because, as they are formed, they are swept away by the flowing liquid and their ions are correspondingly much less abundant. Dynamic FAB spectra are characterized by having far less 'grass' compared with the static mode and therefore sample ions are much easier to observe (the dynamic form of FAB is more sensitive than the static one, even though the basic ionization processes are the same).

Table 1 Some Commonly Used Solvents for FAB or LSIMS

Solvent	Protonated Molecular Ions (m/z)
Glycerol	93
Thioglycerol	109
3-NOBA ¹	154
NOP ²	252
Triethanolamine	150
Diethanolamine	106
Polyethylene glycol (mixtures)	---- ³

1. 3-Nitrobenzyl alcohol
2. n-Octyl-3-nitrophenyl ether
3. Wide mass range depending on glycol used.

Conclusion

By passing a continuous flow of solvent (admixed with a matrix material) from an LC column to a target area on the end of a probe tip and bombarding the target with fast atoms or ions, secondary positive or negative ions are ejected from the surface of the liquid and are extracted into the analyser of a mass spectrometer for measurement of a mass spectrum. As mixture components emerge from the LC column their mass spectra are obtained.

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**Back to Basics Section B:
Interfaces and Ionization Techniques**

CHAPTER B7
PLASMA TORCHES

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Quick Guide

- A plasma consists of a gaseous mixture of neutral species, ions and electrons. The charged species are in approximately equal concentrations.
- For mass spectrometric purposes, the plasma is normally created in argon, a monatomic gas. The plasma then consists of electrons, positive argon ions and neutral argon atoms.
- There are different conditions for producing a plasma, which may be started in gases at low or high (atmospheric) pressures. In a plasma torch, a flow of argon gas is used at atmospheric pressure.
- All methods of plasma production require some electrons to be present as electric discharge initiators. For a plasma torch, the initiating electrons are introduced from a piezoelectric spark directed into argon gas flowing in the interspace of two concentric quartz tubes.
- Near the outlet from the torch, at the end of the concentric tubes, a radio high-frequency coil produces a rapidly oscillating electromagnetic field in the flowing gas. The applied high-frequency field couples inductively with the electric fields of the electrons and ions in the plasma, hence the name 'Inductively Coupled Plasma' or ICP.
- Electrons from a spark are accelerated backwards and forwards rapidly in the oscillating electromagnetic field and collide with neutral atoms. At atmospheric pressure, the high collision frequency of electrons with atoms leads to the electron motion becoming chaotic. The electrons gain rapidly in kinetic energy until they have sufficient energy to cause ionisation of some gas atoms.
- When ionisation occurs, an incident electron collides with an atom, producing a positive ion and second electron. Thus, two electrons appear after collision where there was only one initially.
- The two electrons emerging from the collision are again speeded up until each is able to produce another electron by collisional ionisation of another atom of argon. The process continues so that the first incident electron becomes two, the two become four and so on. This cascade or multiplication increases the number of electrons and ions in the gas to form a plasma within a few milliseconds.

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- The density or numbers of ions and electrons increases quickly in the argon gas, at the same time increasing their kinetic energies as they are pulled back and forth in the applied electromagnetic field and undergo frequent collisions with neutral gas atoms. Some recombination of ions and electrons also occurs to form neutrals.
- An approximate equilibrium is set up in the plasma, with the electrons, ions and atoms having velocity distributions similar to those that would have been the case had the gas been heated thermally to temperatures of 7000–10,000 °C.
- Since the plasma is ignited towards the end of the concentric tubes from which argon gas is issuing, the plasma itself appears as a pale blue to lilac ‘flame’ coming out of the end of the tube. This is the reason for calling the system a ‘torch’, as in a welding torch.
- If a sample is introduced as a solution into the middle of the start of the ‘flame’, the high temperatures and energetic electrons and ions lead to sample molecules being broken down into constituent atoms and their ions. These elemental ions and atoms emerge from the end of the ‘flame’.
- By using a sampling device, the ions are taken off from the end of the plasma flame and led into an ion mass analyser, such as a quadrupole instrument. The abundances of the ions and their m/z values are recorded.
- For several reasons, including the complete breakdown of sample into its substituent elements in the plasma and the use of an unreactive monatomic plasma gas (argon), background interferences in the resulting mass spectra are of small importance. Since there are no or very few background overlaps with sample ions, very precise measurements of sample ion abundances may be made and this allows the determination of precise isotope ratios.
- Inductively coupled plasmas are used to give the ions needed for measurement of either relative concentrations (amounts) of the various elements in a sample or for obtaining accurate elemental isotope ratios.

Summary A discharge ignited in argon and coupled inductively to an external high-frequency electromagnetic field produces a plasma of ions, neutrals and electrons, having a temperature of about 7000 to 10,000 °C. Samples introduced into the plasma under these extremely energetic conditions are fragmented into atoms and ions of their constituent elements. These ions are examined by a mass analyser, frequently a quadrupole instrument.

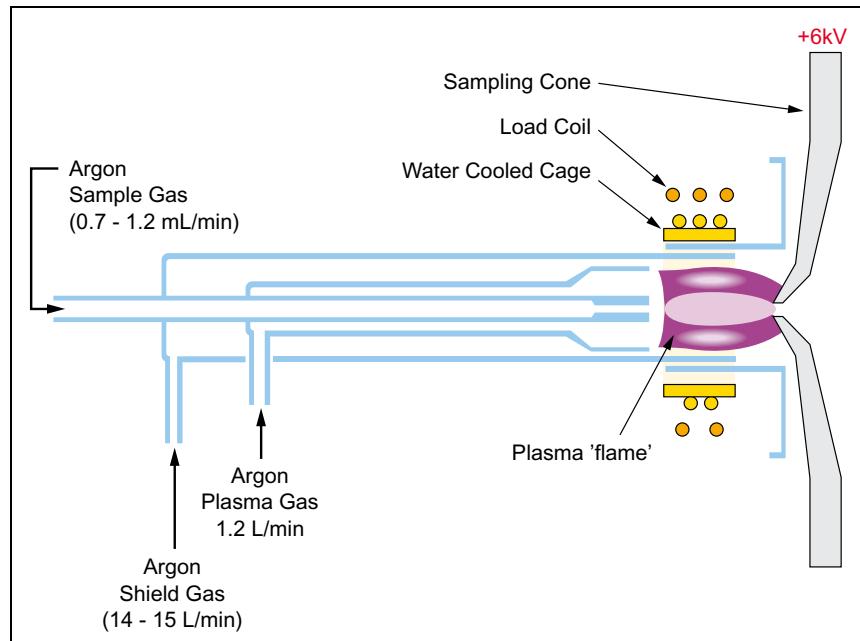


Figure I. Argon gas flows at a rate of about 1–2 L/min along the second of three concentric quartz tubes and is ‘ignited’ to form a plasma by introducing a few sparks from a piezoelectric device. The plasma is maintained and heated by a high-frequency electromagnetic field passing through a ‘load’ coil, which is wound around the outside of the torch. There is an annular space between the load coil and the outermost quartz tube and a water-cooled ‘cage’ (see later) may be placed there. Because the very hot plasma could melt the outermost quartz tube if it impinged on it, a second fast moving coolant argon gas flow is used to separate the plasma itself from the walls of the tube; this ‘shield’ gas flows at a rate of about 14–15 L/min. Finally, there is a third flow of argon through the central quartz tube of about 0.7–1.2 L/min; this gas first passes through the sample, which is carried into the flame. The plasma itself is *relatively* cool at its centre and is mostly heated towards its outside by the high-frequency field. The end of the plasma flame is shown impinging onto the orifice of the sampling cone, which is part of a thick nickel-plated copper disc used to dissipate heat quickly. Electrons, ions and neutrals pass through the sampler cone orifice and into the interface region before mass analysis. The positive ions are accelerated into the interface region by the large positive potential of about 6000 volts and, at the same time, electrons are pulled out and are neutralized at the sampling cone.

PLASMA TORCHES

Introduction

This article should be read in conjunction with the Back-to-Basics guide, *Coronas, Plasmas and Arcs*.

A plasma is defined as a gaseous phase containing neutral molecules, ions and electrons. The numbers of ions and electrons are usually almost equal. In a plasma torch, the plasma is normally formed in a monatomic gas such as argon flowing between two concentric quartz tubes (Figure 1). In the plasma, collisions between atoms, positive ions and electrons leads to their kinetic energies being ‘thermalised’, viz., the distribution of kinetic energies corresponds to that which would be present in a hot gas heated to an equivalent temperature thermally. Direct heating of a gas is not used to form the plasma and, instead, a high-frequency electromagnetic field is applied through the load coil. This rapidly oscillating electromagnetic field interacts inductively with the charged species; electrons and ions try to follow the field and are speeded up. The electrons and ions gain kinetic energy. In the rapidly oscillating field, random collisions of electrons and ions with neutral species causes this extra kinetic energy to be redistributed and the whole ensemble becomes hotter. If the rapidly oscillating electromagnetic field is maintained, the ions and electrons continue to follow a chaotic motion as they become faster and faster and yet undergo more and more collisions. These collisions continue to redistribute the kinetic energies. Eventually, the kinetic energies become so high that the plasma may reach a temperature of up to about 10,000 °C but more normally it is about 8000 °C. At these sorts of temperatures, the plasma behaves like a flame issuing from the ends of the concentric quartz tubes, hence the derivation of the name ‘plasma torch’. As in a normal flame, atoms and ions are excited electronically in the collision processes and emit light. In the plasma torches used for mass spectrometry, the argon, which is used, emits pale blue to lilac light as excited atoms relax and as a proportion of the electrons and ions recombine.

If a sample solution is introduced into the centre of the plasma, the constituent molecules are bombarded by the energetic atoms, ions, electrons and even photons from the plasma itself. Under these vigorous conditions, sample molecules are both ionised and fragmented repeatedly until only their constituent elemental atoms or ions survive. The ions are drawn off into a mass analyser for measurement of abundances and m/z values. Plasma torches provide a powerful method for introducing and ionising a wide range of sample types into a mass spectrometer (Inductively Coupled Plasma Mass Spectrometry, ICPMS).

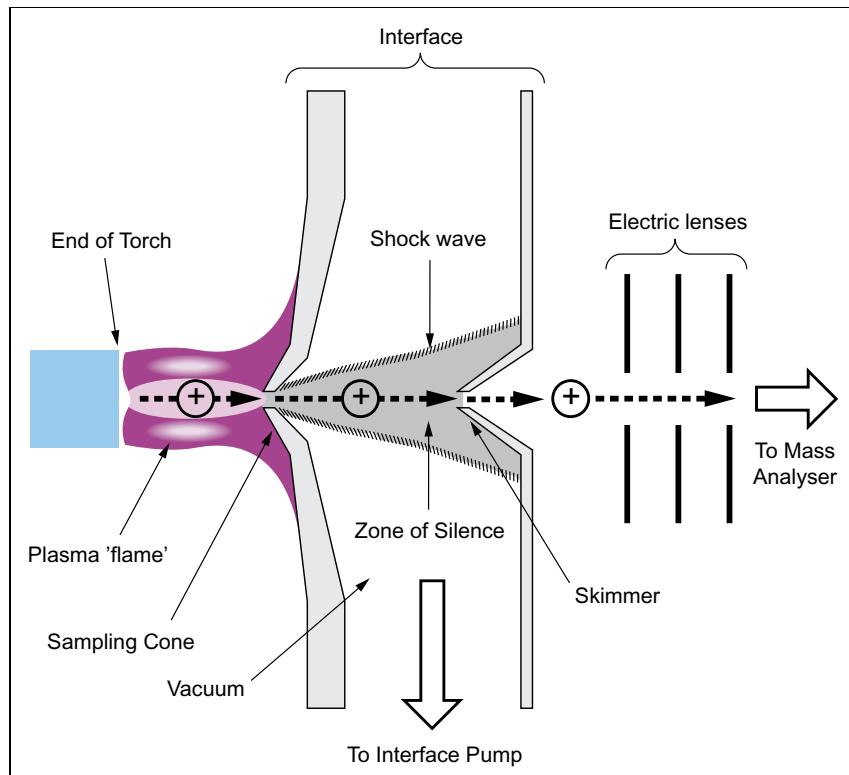


Figure 2. Hot ions and atoms formed in the plasma pass through the sampling cone orifice and into a vacuum of about 10^{-5} Torr produced between it and the skimmer by an interface pump. Electrons are captured by the sampler, which is held at +6000 V. As the gases expand rapidly into the evacuated space, they produce a shock wave in the form of a hollow cone. The required ions are in the centre of this cone (called the 'Zone of Silence'), forming a supersonic jet travelling at about 1000 m/s. The beam of ions passes through a second orifice (the skimmer) and into the mass analyser. There is usually a system of lenses after the skimmer to collimate the ion beam before it enters the analyser itself. The passage of the positive ions is indicated (---). There may be a direct line-of-sight from the plasma flame to the ion collector and the emitted light would strike the ion collector, producing a spurious background current. To prevent this, a small metal plate (the 'light stop') is inserted into the line-of-sight but then the ion beam must be deflected around it before going on to the analyser. Alternatively, the ion beam can be deflected away from the 'line-of-sight' via a hexapole and into the analyser.

Because light emitted from inductively coupled plasma torches is characteristic of the elements present, the torches were introduced originally for instruments that optically measured the frequencies and intensities of the emitted light and used these, rather than ions, to estimate the amounts and types of elements present (Inductively Coupled Plasma Atomic Emission Spectroscopy, ICPAES). The mass spectrometric approach has introduced a wider ranging and more sensitive system for estimation of element types and abundances in a huge range of sample types. More details of the plasma torch follow.

Construction of the plasma torch

Figure 1 illustrates a typical arrangement for construction of a plasma torch. Essentially, it consists of three concentric quartz tubes. Argon gas flows through all three tubes. The middle tube is used to start the plasma, with the shield gas flowing to prevent the plasma from impinging onto the walls of the outer tube. The innermost tube is used for the introduction of the sample into the centre of the plasma flame. The end of the torch is surrounded with a few turns of copper coil, which carry the high-frequency (27 or 40 MHz) electromagnetic field, which couples inductively with charged species in the plasma.

When the plasma has formed at the exits to the concentric tubes, it assumes a shallow, rounded cone shape with a hollow centre, into which is introduced the sample. The back of the plasma is prevented from touching and melting the inner concentric tubes by adjusting the flow of argon to a sufficiently high rate that the plasma is not fully developed until it is almost into the coil region (Figure 1). In some torches, there may be also a concentric water-cooled ‘cage’, lying outside the outermost quartz tube but inside the high-frequency copper coil. The cage leads to cooler flames and to improved performance in some applications; the use of a cage is discussed later.

The end or front of the plasma flame impinges onto a metal plate (the ‘cone’ or ‘sampler’ or ‘sampling cone’) having a small hole in its centre (Figure 2). The region on the other side of the cone from the flame is under vacuum and therefore the ions and neutrals passing from the atmospheric pressure hot flame into a vacuum space are accelerated to supersonic speeds and cooled as rapid expansion occurs. A supersonic jet of gas passes towards a second metal plate (the skimmer) containing a smaller hole than the one in the sampler, where ions pass into the mass analyser itself. The sampler and skimmer form an interface between the plasma flame and the mass analyser. A ‘light stop’ must be used to prevent photons from the plasma flame reaching the ion collector since this would produce a spurious high background signal.

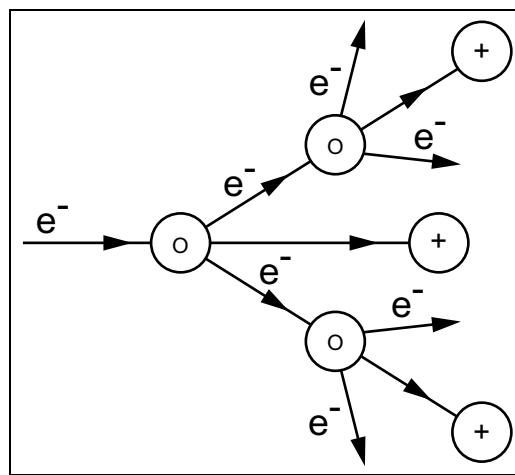


Figure 3. In a cascade process, one incident electron (e^-) collides with a neutral atom (\odot) to produce a second electron and an ion ($+$). There are then two electrons and one ion. After these two electrons have each collided with another neutral atom, there are produced four electrons and three ions. This process continues and, after about 20 successive sets of collisions, millions of electrons and ions will have been formed rapidly (the mean free path between collisions is very small at atmospheric pressures). A typical atmospheric pressure plasma will contain 10^{16} each of electrons and ions per millilitre. Some ions and electrons are lost by recombination to reform neutral atoms, with emission of light.

Further aspects of the torch and the interface are described below.

The plasma flame

The argon gas flowing through the concentric quartz tubes shown in Figure 1 and through the high-frequency field does not become a plasma until a few electrons have been introduced near the flame end of the concentric tubes. The following sequence of events occurs within a few milliseconds. A hot spark, usually produced piezoelectrically, contains electrons and, as these are carried by the flowing argon gas they enter the oscillating high-frequency electromagnetic field, where they are accelerated rapidly back and forth by its changing magnetic and electric components. At the same time, the electrons collide with neutral argon atoms so that, together with the oscillating field, their motions become chaotic. Nevertheless, the electrons continue to be accelerated until they gain enough energy to cause ionisation of some argon atoms. At this crucial stage, more electrons and ions are produced in a cascade process (Figure 3) so that within a few milliseconds a high concentration of ions and electrons is produced in the flowing argon gas. This is the plasma, which glows with light emitted from excited atoms and ions and from recombination of electrons with ions (see Back-to-Basics guide on *Coronas, Plasmas and Arcs*). This glow gives the argon plasma its characteristic pale blue to lilac coloration. There are approximately equal numbers of positive ions and electrons in a plasma and therefore not much space charge. The numbers of ions and electrons reaches a number density of about 10^{15} to 10^{16} per millilitre.

Because the flowing argon gas passes through the high-frequency electromagnetic field placed at the ends of the concentric tubes, the plasma actually appears as a 'flame' near the end of the 'torch', residing inside the outermost concentric tube. By arranging suitable argon gas flows through the three tubes, the very hot plasma flame is prevented from actually contacting the three tubes, which would otherwise melt. The plasma is selfsustaining in that there is no further need to seed it with electrons, sufficient of the latter being around in the plasma itself to ensure that the process continues until either the gas flow or the high-frequency field is switched off. As the flame is formed mostly from the gas flowing through the second of the three concentric tubes, there is a small 'hollow' space at its centre due to the presence of the innermost tube, from which a cool flow of sample entrained in argon gas enters and mixes with the flame (Figure 2). The outer 'skin' of the plasma is very hot because this is where most of the high-frequency heating takes place and, to shield the outermost concentric quartz tube (Figure 1) from this very hot region of the flame, there is a third flow of argon, which serves to shield the walls of the tube by sweeping the plasma flame away from them. This flow

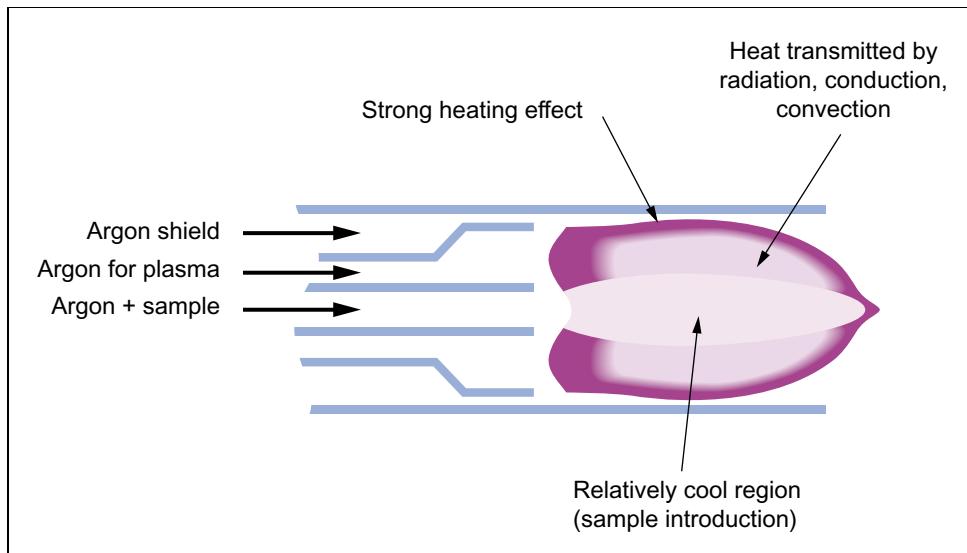


Figure 4. In the plasma flame, much of the heating effect of the high-frequency electromagnetic field occurs in the outer 'skin' of the flame, in common with all high-frequency electromagnetic heating. The hot electrons, ions and neutrals mingle with cooler materials towards the centre of the flame and energy is transferred by conduction, convection and radiation. The inner regions of the flame are somewhat cooler than the outer but they are still very hot! At the centre of the flame, there is a flow of cold argon gas, carrying the sample so that this region is the coolest. At the outer periphery of the flame, coolant argon gas is used to prevent the very hot plasma from impinging on and melting the outer quartz tube. The rapidly flowing argon gas carries the really hot parts of the plasma beyond the ends of the concentric tubes so that the latter do not become too hot. The three gas flows are shown on the diagram, together with an indication of the hotness of the flame in different regions. From hottest to coolest, the temperature ranges between about 7000–8,000 and 4000–5000 K but these ranges depend a great deal on the actual conditions of argon gas flow and power input from the high-frequency field.

of cold argon also serves to cool the outermost tube as well as protect it from the flame. The uses and effects of the three gas flows are illustrated further in Figure 4.

The temperature of the plasma

If a gas is contained in a vessel and then heated thermally, the constituent atoms of the gas gain thermal energy on striking the hot walls of the containment vessel. The heat energy transferred to molecules increases their thermal motions (rotation, vibration, translation). Under equilibrium conditions, the added thermal energy is distributed amongst the three modes but, for monatomic gases, all of this increase in energy appears as kinetic (translational) energy because there are no bonds to vibrate or rotate in atoms. Thus, the process of heating a gas 'thermally' is characterised by increased kinetic energies of its constituent atoms or molecules. Atomic or molecular mass is fixed so that the increased kinetic energy ($= mv^2/2$) appears as increased velocity of motion, viz., as the gas gets hotter, its constituent atoms or molecules move faster. As the atoms get faster and collide with other atoms, thermal energy is shared at each collision and so 'hot' atoms or molecules pass on some of their kinetic energy to other cooler atoms or molecules. The total kinetic energy added through heating becomes distributed throughout the bulk of the gas. At atmospheric pressure, the mean free path between collisions of atoms or molecules is very short and so the extra energy is rapidly distributed through simple collision mechanisms. However, at atmospheric pressures, bulk movement of gas (convection) also leads to mixing. Finally, some of the heating effect arises directly through absorption of radiant energy from outer hotter gases. Thus, at any one temperature attained by a gas, its atoms or molecules have a distribution of kinetic energies, which is characterised by the Boltzmann equation *if the gas has attained equilibrium conditions* (Figure 5). To obtain a temperature of 8000 to 10,000 K by externally heating a gas, the walls of the containment vessel would have to be heated to an even higher temperature but there are no materials that can withstand such temperatures.

In a plasma, the constituent atoms, ions and electrons are made to move faster by an electromagnetic field and not by application of heat externally or through combustion processes. Nevertheless, the end result is the same as if the plasma had been heated externally, viz., the constituent atoms, ions and electrons are made to move faster and faster, eventually reaching a distribution of kinetic energies, which would be characteristic of the Boltzmann equation applied to a gas that had been heated externally. In other words, the electromagnetically heated gas attains a near equilibrium condition, which is equivalent to some temperature T that might have been achieved thermally. The plasma can be described as having a

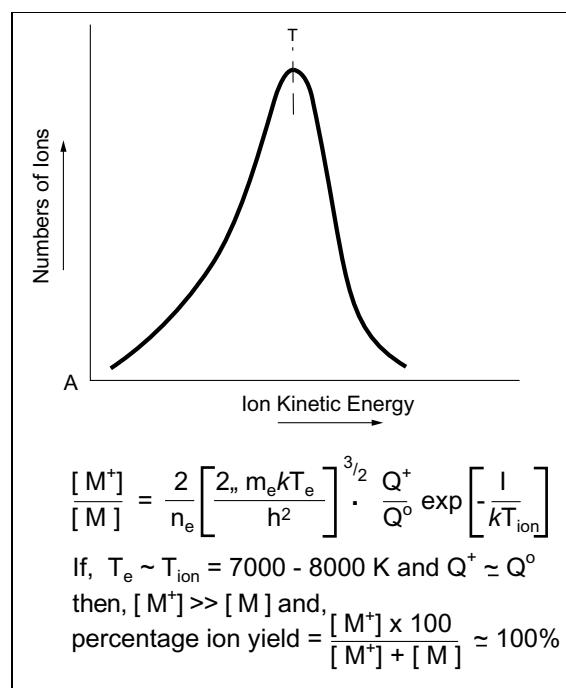


Figure 5. At equilibrium, at some temperature T , the atoms or molecules of a gas will have a range of kinetic energies, characteristic of the temperature. The diagram illustrates this for one temperature. It can be seen that, at one end of the curve, there is a fairly sharp cut-off in numbers of atoms or molecules having high kinetic energies. At the other end, there is a more gradual drop in numbers of molecules having lower energies. The position of the top of the peak is a characteristic of the temperature and moves from left to right as temperature increases.

The equation illustrates how the number of singly-charged positive ions, $[M^+]$, in relation to the number of neutral particles, $[M]$, may be calculated. For example, with potassium at 8000 K, the ratio of ions to neutral atoms in a plasma is about 2000:1. Thus, at high temperatures, $[M^+] \gg [M]$ and the yield of ions reaches close to 100% for all elements. This means that high sensitivities for detection of ions depends partly on having a high plasma flame temperature. However, at the higher temperatures, new 'molecular ions' such as ArO^+ begin to form and, to suppress these, it may be necessary to operate the flame at a lower temperature. [T_e = electron temperature, T_{ion} = ion temperature, m_e = the rest mass of an electron, k = the Boltzmann constant, h = Planck's constant, Q^+ , Q° = partition functions (approximately equal), I = ionisation energy for the element considered.]

temperature T defined by the speed of movement of its constituent atoms, ions and electrons. For an atmospheric pressure plasma struck in argon, the kinetic energies of the ions, atoms and electrons become such as to be equivalent to having heated the gas thermally to about 6000–8,000 K, depending on the conditions used. A plasma flame may be characterised by its temperature, although there are some other details that need to be considered.

The first arises because of the three species present (atoms, ions and electrons). The electrons have very small mass compared with the atoms and ions and can be accelerated more easily by the electromagnetic field than can the ions. Thus, the electrons move very much faster than the ions and can be said to have a higher temperature (kinetic energy) than do the ions. In turn, the movement of ions is affected by the electromagnetic field but not that of atoms. The ions move somewhat faster than the atoms and the temperature of the ions can be described as being higher than that of the atoms. The atoms gain in velocity through collisions with hot ions and electrons. Therefore, unlike a gas heated externally and having mostly only atoms or molecules present, a plasma contains three species, each with its own ‘temperature’ and $T_e > T_i > T_a$, where T_e is the mean temperature of electrons, T_i that of ions and T_a that of atoms. Often T_i and T_a are very similar and are simply described as a gas temperature, T_g . T_e normally lies in the range of 6000–8,000 K and T_g in the range 5000–7000 K. Due to convective, conductive and radiative effects, these temperatures tend to be equalised and it is often sufficiently accurate for many purposes to describe the plasma flame as having a single temperature.

The second limitation arises from the method of heating. For a contained gas heated externally, rapid collisions and convection currents mean that the temperature throughout the gas rapidly becomes uniform (it is said to be equilibrated). A plasma heated by inductive coupling to a high-frequency electromagnetic field is not heated uniformly. Typically for high-frequency heating of any material a ‘skin’ effect is found and most of the direct heating occurs near the surface of the plasma (Figure 4). However, this heat soon becomes distributed to the remainder of the flowing gas through diffusion, convection, conduction and radiation. As the gas is flowing, there is insufficient time for the skin heating to attain full equilibration conditions so that there is a temperature profile from hot to cooler in going from the outside to the inside of the plasma. The central regions of the plasma, into which sample is introduced, are somewhat cooler than the outer reaches, but still hot.

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A calculation of ion yields is shown in figure 5. The higher the temperature, the greater the degree of ionisation so that, at 6000–8000 K, most atoms exist as ions in the plasma. Thus, for most elements in the plasma, ionisation efficiencies reach to over 90–95%. Operation of the plasma torch under conditions that produce a cooler flame ('cold plasma') has important advantages for some applications, which are discussed after the next section.

Processes occurring in the plasma after introduction of a sample

The formation of ions and electrons in a neutral gas so as to give the plasma itself has been described above. If a sample substance is introduced into the plasma, its constituent molecules experience a number of radiative, convective and collisional processes that lead to the molecules being completely broken down into their constituent atoms, which appear mostly in ionised form.

Any sample to be introduced into the centre of the plasma flame is often first nebulised (broken down into small droplets) by using argon gas as a 'spraying' medium. The argon gas and the spray of droplets flow down a central tube of the plasma torch (Figure 1 and 4) and into the centre of the plasma flame. The droplets rapidly lose lower boiling solvent molecules at the high temperatures in the plasma and solvent and solute molecules diffuse into the plasma itself, where a number of processes occur leading to molecular fragmentation.

Fast moving high temperature electrons in the plasma collide with sample molecules and cause ionisation (Figure 6); the energy transferred in this process also causes some fragmentation. More collisions of the products from this first step with electrons cause more extensive fragmentation. Additionally, collisions of fragments or intact molecules with fast moving 'hot' ions and atoms from the plasma lead to the transfer of thermal (rotational and vibrational) energy, which also gives rise to thermal 'cracking' of sample molecules. The 'cracked' fragments are themselves subjected to collision with electrons, ions and atoms and are broken down and ionised even more. The numbers of such collisions and the total energy transferred during the time the sample molecules are in the plasma is so high that, within about 2–3 milliseconds, every sample molecule is broken down into its constituent atoms and most of these have been ionised. Essentially, it can be said that, at the high temperatures within the plasma, sample molecules are rapidly torn apart into ions of their constituent elements. Conditions inside the plasma are so energetically fierce that all kinds of sample molecules are literally shredded into their constituent elements. If the elements appear as excited atoms, emission of light occurs and this can be used to examine the elemental composition of a sample by using a

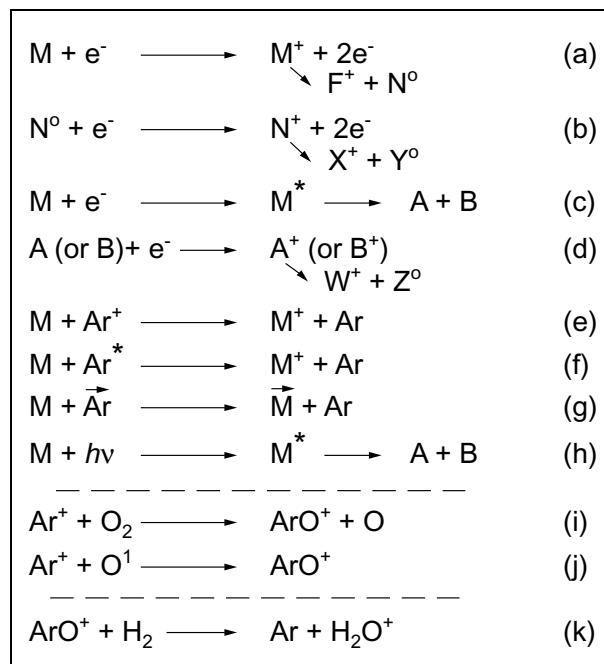


Figure 6. The various reactions shown illustrate some of the major routes to fragmentation of molecules inside a plasma.

- (a) Ionisation and fragmentation following collision of a molecule M with an electron to produce molecular ions M^+ , fragment ions F^+ and neutrals N° .
- (b) Ionisation and fragmentation of neutrals N° from (a) by collision with other electrons to give ions N^+ , X^+ and neutrals Y° .
- (c) Vibrational excitation of molecules M by electrons to give M^* followed by bond breaking to give neutral fragments A,B.
- (d) Ionisation of neutrals A, B from the latter process.
- (e) Charge exchange of an argon ion and a molecule M.
- (f) Ionisation by collision of an excited argon atom with a molecule M.
- (g) An illustration of a ‘fast’ argon atom colliding with a molecule M and passing on some of its kinetic energy (designated by the arrow).
- (h) Absorption of radiation $h\nu$ leading to bond breaking in the molecule M after excitation to M^* .
- (i,j) In the lower part of the above scheme are shown two ways in which ArO^+ may be formed by collisional processes.
- (k) Collision of ArO^+ ions with H_2 to form Ar^+ and H_2O .

spectrophotometer to sample the light emitted (ICPAES), by which it identifies elements from their characteristic atomic emission lines and the amounts of the elements from the intensities of the emission lines. However, a very large proportion of the elements appear as ions, which are best examined by mass spectrometry (ICPMS). The ions are mostly singly charged but, depending on ionisation energies and the temperature of the flame, there are also doubly charged ions.

It may be noted that the plasma emits light of high intensity inside the plasma itself and over a wide spectrum of wavelengths and some of this light is absorbed by the sample molecules or their fragments and is rapidly converted into internal vibrational and rotational energy. This extra energy may itself lead to fragmentation of the sample molecules or of the fragments already produced from them by other processes.

In addition to the fragmentation reactions, there are also some interactions of various species, which lead to the synthesis of unexpected and unusual 'molecular ions'. For example, the argon gas of the plasma can combine with oxygen to give the relatively stable ArO^+ ions, or with hydrogen to give ArH^+ . These 'unexpected' ions have relatively strong ionic bonds and are formed at the high temperatures within the plasma. Their masses coincide with (are *isobaric* with) isotopes of other elements and are said to interfere with the latter. For example, ArO^+ (mass = 56) is isobaric with the main isotope of iron (mass = 56) and ArH^+ (mass = 41) is isobaric with an isotope of potassium. One way of reducing the numbers of these unwanted so-called molecular ions is to run the plasma under somewhat cooler conditions, when it is known as a 'cold plasma'. This term is relative because the plasma still reaches temperatures of several thousand degrees.

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Cold plasma conditions and the plasma cage

Although the plasma has been described as producing a very low background of extraneous ions, the interfering molecular ions discussed above are produced in sufficient quantities as to lead to difficulties in accurate measurement of isotopic ratios for certain elements. The plasma gas itself produces Ar^+ ($m/z = 40$, which interferes with calcium), ArH^+ ($m/z = 41$; interferes with calcium) and Ar_2^+ ($m/z = 80$, interferes with selenium). Other typical interfering ions arise from the solvents and give O^+ , NO^+ , ArO^+ , O_2^+ and so on. Under 'cold plasma' conditions, the abundances of these interfering isobaric ions can be reduced by several orders of magnitude.

There are several methods of producing cold plasmas. For example, the power input from the coil may be reduced and the sample injector gas flow increased. Reducing the power reduces the heating effect but still produces energetic electrons and ions. Increasing the gas flow to the inner regions of the plasma via the sample inlet causes a temperature reduction through mixing more cold gas with the plasma gas. Similarly, some of the interfering molecular ions can be reduced in amount simply by introducing another gas into the system, as with the inclusion of some nitrogen into the argon gas flow.

An important means of producing a cold plasma is to place a 'cage' or 'shield' not quite all around the outermost tube of the plasma gun but inside the high-frequency coil (Figure 1). The shielding leads to greatly reduced penetration of the electromagnetic field into the interior of the plasma. Thus, the outermost 'skin' heating takes place but electromagnetic heating within the body of the plasma from the high-frequency field is reduced, although this region still receives heat from mixing (conduction, convection and radiation) and is still quite hot at 4500 to 5000 K.

Although cold plasmas have benefits in removing interfering ions such as ArO^+ , they are not necessary for other applications where interferences are not a problem. Thus, in laboratories where a range of isotopes needs to be examined, the plasma has to be changed between 'hot' and 'cold' conditions, whereas it is much simpler practically if the plasma can be run under a single set of conditions. For this reason, some workers use 'warm' plasmas, which operate between the 'hot' and 'cold' conditions.

The 'cold' plasmas tend to be unstable, are sometimes difficult to maintain and the total yields of ions are less than those of the hot plasmas. To obviate the difficulties of the interfering isobaric molecular ions from hot plasmas, it has been found highly beneficial to include a collision cell (hexapole, see Back-to-Basics guide on *Quadrupoles*) before the mass analyser itself. This collision cell contains a low pressure of hydrogen gas. Ion/molecule collisions

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between the hydrogen and, say, ArO^+ lead to the removal of the latter through formation of neutral Ar atoms and H_2O^+ (Figure 6k). This principle can be extended into the flame itself. For example, C^+ ions formed from carbonaceous materials in the flame interact with Se atoms to give Se^+ ions and C atoms and this indirect process enhances the yield of Se^+ ions formed in the flame directly.

The interface

Ions produced in the plasma must be transferred to a mass analyser. The flame is very hot and at atmospheric pressure but the mass analyser is at room temperature and under vacuum. To effect transfer of ions from the plasma to the analyser, an interface must be used which needs to be as efficient as possible if ion yields from the plasma are to be maintained in the analyser.

A typical interface is shown in Figure 2. The orifice in the sampler must be large enough to sample the centre of the plasma flame while disturbing the plasma as little as possible. A diameter of about 1 mm is used normally, this being well in excess of the Debye length (Λ_D) but not so large as to let too much plasma gas or even air into the interface region as this would make difficult the maintenance of a suitable vacuum behind the sampler. The Debye length is a measure of the width of the effective electric field of an ion and is given approximately by the formula (1), in which T_e is the electron temperature and N_e is the number density of electrons (per mL).

$$\Lambda_D = 6.9(T_e/N_e)^{1/2} \quad (1)$$

For a plasma temperature of 8,000 K and $N_e = 10^{14}/\text{mL}$, Λ_D is about 0.0006 mm, which is very much smaller than the 1mm sampler orifice and so ions can pass through easily. Hot gases from the plasma impinge on the edges of the sampler orifice so that deposits build up and reduce its diameter with time. The surrounds of the sampler orifice suffer also from corrosive effects due to bombardment by hot species from the plasma flame. These problems necessitate replacement of the sampler from time to time.

As the gas leaves the other side of the sampler orifice, it experiences a vacuum of about 10^{-5} Torr and the expanding jet of gas cools very rapidly and reaches supersonic speeds. There is a resulting conical shock wave, inside which is the 'zone of silence' (Figure 2). Air and other gases diffuse from outside and into the zone of silence and the amount eventually reaches a level at which the shock wave heats it up along a frontal zone called the 'Mach disc'. Therefore, to sample the fast moving ions issuing from the sampler, the entrance to the next orifice (the skimmer) needs to be sited within the zone of silence and

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before the Mach disc, which means it is normally placed about 5–6 mm from the sampler. The skimmer orifice needs to be small enough that it prevents too much exterior neutral gas getting through it and into the analyser region and yet large enough that it exceeds the mean free path of the particles passing through it so as not to interrupt the flow. For these reasons, the skimmer orifice is about 0.8 mm in diameter.

After the skimmer, the ions must be prepared for mass analysis and, in front of the analyser itself, electronic lenses are used to adjust ion velocities and flight paths. The skimmer can be considered to be the end of the interface region stretching from the end of the plasma flame. Some sort of 'light stop' must be used to prevent emitted light from the plasma reaching the ion collector in the mass analyser (Figure 2).

Conclusion

A plasma of electrons, ions and neutrals produced in gas flowing through concentric tubes is maintained and heated to some 5000 - 8000 K by inductive coupling to a high (radio)frequency electromagnetic field. Sample substances introduced into the hot plasma are torn apart into their constituent atoms, most of which are ionised. The ions are measured for abundance and m/z values by a suitable mass analyser, often a quadrupole or time-of-flight instrument.

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**Back to Basics Section B:
Interfaces and Ionization Techniques**

**CHAPTER B8
NEBULIZERS**

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Quick Guide

- Samples to be examined by ICP-MS are frequently in the form of a solution of an analyte in a solvent, which may be aqueous or organic.
- For mass spectrometric ionisation and introduction into a plasma flame, the analyte needs to be separated from most of the accompanying solvent. For this purpose, the solution is first broken down into small droplets through use of a nebulizer.
- Nebulizers convert bulk liquid into an aerosol, consisting of a mix of small droplets of various sizes and solvent vapour. Such devices are used for transferring analyte solutions into the flame of a plasma torch.
- The aerosol is swept to the torch in a stream of argon gas. During passage from the nebulizer to the plasma flame, the droplets rapidly become smaller as solvent evaporates and eventually become very small. In many cases, almost all of the solvent evaporates to leave “dry” particulate matter of residual analyte.
- To assist evaporation of solvent, the argon stream carrying the aerosol may be passed through a heated tube called a desolvation chamber, operated at temperatures up to about 150 °C.
- The large quantities of solvent vapour produced from the evaporating droplets must be removed before reaching the plasma flame and this is done by having cooling tubes sited after the heated desolvation chamber to condense the vapour into liquid. This condensed liquid is run to waste.
- After desolvation, the remaining very fine particulate matter and residual droplets are swept by the argon carrier into the plasma flame, where fragmentation and ionisation occur.
- Depending on the type of nebulizer used and its efficiency, there may be initially a significant proportion of large droplets in the aerosol. Being heavier than the very fine droplets, the larger ones are affected by gravity and by turbulent flow in the argon sweep gas, which causes them to deposit onto the walls of the transfer tube.
- To assist in the deposition of these larger droplets, nebulizer inlet systems frequently incorporate a “spray chamber” sited immediately after the nebulizer itself and before the desolvation chamber. Any liquid deposited in the spray chamber is wasted analyte solution, which may be run off to waste or may be recycled.

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- A nebulizer inlet may consist of, (a) only the nebulizer, (b) a nebulizer and a spray chamber or, (c) a nebulizer, a spray chamber and a desolvation chamber. Whichever arrangement is used, the object is to transfer analyte to the plasma flame in as fine a particulate consistency as possible, with as high an efficiency as possible.
- The efficiencies of transfer of analyte solution from the nebulizer to the plasma flame depend very much on nebulizer design and vary widely from about 5–20% and sometimes almost up to 100%.
- There is a very wide range of designs for nebulizers but most are based on some form of gas/liquid sprayer or on ultrasonics.
- In the gas/liquid spray form of nebulizer, a stream of gas interacts with a stream of liquid. Depending on the relative velocity of the two streams and their relative orientation, the liquid flow is broken down into a spray of droplets, as in the common hair sprays.
- For ultrasonic nebulizers, the liquid is fragmented into droplets by an acoustic standing wave, usually produced by a piezoelectric transducer.

Summary

For mass spectrometric analysis of an analyte solution using a plasma torch, it is necessary to break down the solution into fine droplet form, which can be swept into the flame by a stream of argon gas. On the way to the flame, the droplets become even smaller and may eventually lose all solvent to leave dry analyte particulate matter. This fine residual matter can be fragmented and ionised in the plasma flame without disturbing its operation.

For a droplet of initial radius r_0 , its radius r_t at time t may be estimated from equation (1), the various terms of which are listed below.

$$r_0^3 - r_t^3 = 980 \times D \cdot \sigma \cdot \Delta p (M/d \cdot R \cdot T)^2 \times t$$

D = diffusivity of the solvent vapour from the sample solution ($\text{cm}^2 \cdot \text{s}^{-1}$)

σ = surface tension of sample solution ($\text{erg} \cdot \text{cm}^{-2}$)

p = vapour pressure of sample solution ($\text{mm Hg} \times 1.359 = \text{g} \cdot \text{cm}^{-2}$)

M = relative molecular mass of the liquid (solvent; $\text{g} \cdot \text{mol}^{-1}$)

d = density of sample solution ($\text{g} \cdot \text{cm}^{-3}$)

T = absolute temperature of droplet (K)

R = gas constant = 8.31×10^7 ($\text{erg} \cdot \text{K}^{-1} \cdot \text{mol}^{-1}$)

The factor of 980 changes g.cm units into ergs.

With typical values for water at 20 °C, the time taken for a droplet to shrink to 10% of its original radius may be calculated as an example of the use of equation (1).

With $D = 0.24$, $\sigma = 73$, $p = 15.5 \text{ mm Hg}$, $M = 18$, $d = 1$, $T = 293 \text{ K}$ then, if $t_t = 0.1 \times t_0$.

$$t_0^3 - t_t^3 = 0.999 \times t_0^3 = 0.224 \times 10^{12} \times t$$

$$\therefore t = \underline{4.5 \text{ s}}$$

At 40°C, assuming the other terms do not change too much, $p = 55 \text{ mm Hg}$ and,

$$t = \underline{1.5 \text{ s}}$$

Let the argon sweep flow of the aerosol through a tube of 1 cm radius be 1 L/min.
In 1.5 s, the argon will flow through a distance (l) given by the formula:

$$l = \frac{\text{flow rate} \times \text{time}}{\text{cross-sectional area}} = \underline{7.9 \text{ cm}}$$

Figure 1. The calculation shows how rapidly a droplet changes in diameter with time as it flows towards the plasma flame. At 40 °C, a droplet loses 90% of its size within about 1.5 seconds, in which time the sweep gas has flowed only about 8 cm along the tube leading to the plasma flame. Typical desolvation chambers operate at 150 °C and, at these temperatures, similar changes in diameter will be complete in times of a few milliseconds. The droplets of sample solution lose almost all of their solvent (dry out) to give only residual sample (solute) particulate matter before reaching the plasma flame.

NEBULIZERS

- Background** For use in ICP-MS, it is necessary to transport solutions of analyte into the plasma flame. The thermal mass of the flame is small and ingress of relatively large quantities of extraneous matter such as solvent would cool the flame and might even put it out altogether. Even cooling the flame reduces its ionisation efficiency with concomitant effects on the accuracy and detection limits of the ICP-MS method. Consequently, it is necessary to remove as much solvent as possible. This may be done by evaporation off-line or it may be done on-line by spraying the solution as an aerosol into the plasma flame.
- The nature of an aerosol** If a liquid is vaporised rapidly or its vapour is cooled rapidly, it may form an aerosol, which consists of a mixture of purely gaseous components, small droplets and, sometimes, small particles of solid matter (particulates). Aerosols are dynamic systems, with evaporation from some droplets that become smaller and coalescence of other droplets that grow bigger. Given time and suitable temperatures, the components of aerosols may condense to form a liquid and/or may evaporate to form vapour. In the form of a mist of fine droplets, this condensation is not fast, even with strong cooling and an aerosol produced in a gas stream can be swept for quite long distances without serious losses of components of the aerosol. For example, clouds formed in the atmosphere by rapid cooling of warm moist air consist of small droplets of water, which scatter light and hence give rise to the typical opacity of clouds; a similar effect near ground level at low temperatures gives rise to fog or mist. A good example of man-made aerosols are the clouds of water vapour formed above the cooling towers of power stations. Such clouds as well as natural ones are swept along by the wind. Given the right cooling conditions, the small water droplets can coalesce to form larger drops, which eventually may be large enough to fall as rain. Under warmer conditions, the small droplets evaporate completely and the clouds disperse. An aerosol produced instrumentally has similar properties, except that the aerosol is usually produced from solutions and not from pure liquids. For solutions of analytes, the droplets consist of solute and solvent, from which the latter can evaporate to give smaller droplets of more and more concentrated solution (Figure 1). If the solvent evaporates entirely from a droplet, the desolvated ("dry") solute appears as small solid particles, often simply called particulate matter.

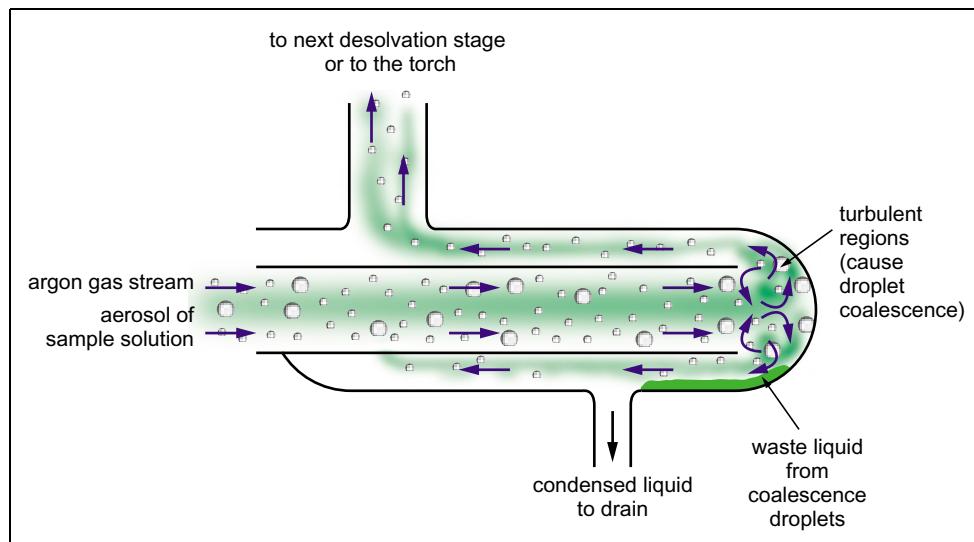


Figure 2. The aerosol, consisting of solvent vapour and droplets having a range of diameters immediately after formation may be passed through a spray chamber (which may or may not be present in any one apparatus). In the spray chamber, a combination of gravity and turbulent flow leads to the larger droplets hitting the walls of the chamber and sticking there. The deposited droplets coalesce into a liquid waste stream, which is drained away. The smaller droplets of the aerosol do not fall out of the gas stream and are swept along to the next stage, which may be the plasma flame but may be a desolvation chamber placed before the flame.

Droplets coming from the spray chamber or direct from the nebulizer become smaller through evaporation of solvent as they flow along in the carrier gas stream. To assist this process, the gas stream is passed through a heating chamber held at about 150 °C, which leads to much more rapid desolvation of the droplets. It is preferable to remove most or all of the solvent vapour that has been produced before it reaches the plasma flame. To do this, a cooling chamber follows the hot desolvation chamber and induces the solvent to condense. This condensate of solvent flows to waste and residual “dry” particulate matter is swept into the plasma flame by the argon gas flow.

Aerosols may be produced as a “spray” of droplets by various means. A good example of a nebulizer is the common household hair spray, which produces fine droplets of a solution of hair lacquer by using a gas to blow the lacquer solution through a fine nozzle so that it emerges as a spray of small droplets. In use, the droplets strike the hair and settle and the solvent evaporates to leave behind the involatile lacquer itself. For mass spectrometry, a spray of a solution of analyte can be produced similarly or by a wide variety of other methods, many of which are discussed here. The Back-to-Basics guides *Thermospray*, *APCI* and *Electrospray* contain details of droplet evaporation and formation of ions of relevance to this present guide. Aerosols are also produced by laser ablation for which the relevant Back-to-Basics guide should be consulted.

The term “nebulizer” is used generally as a description for any spraying device, such as the hair spray mentioned above. It is normally applied to any means of forming an aerosol spray, in which a volume of liquid is broken up into a mist of vapour and small droplets and possibly even solid matter. There have been many forms of nebulizer designed for transmitting a solution of analyte in droplet form to a plasma torch in ICP–MS and to the inlet/ionisation sources used in ES–MS and APCI–MS.

General principles of aerosol formation

For use in ICP–MS, an aerosol of analyte solution is produced in a nebulizer by mechanically breaking up the solution into a spray of droplets and solvent vapour. This spray is swept along to the plasma flame by a flow of argon gas. The droplets have a range of diameters, which depends on the type of nebulizer used. Frequently, before entering the torch, the aerosol first passes through a “spray chamber”, designed to remove many of the larger droplets through their collision with the walls of the spray chamber under the influence of turbulence and gravity (Figure 2). During passage to the flame, all of the droplets lose solvent by evaporation (“desolvation”) becoming smaller as they do so (Figure 1). To remove more solvent, the spray may be passed through a “desolvation chamber”, a section of tubing heated to about 150 °C, which is followed by a cooling section to condense out the solvent vapour formed on evaporation. This condensed solvent vapour is allowed to drain away. Before entering the flame, the residual spray may not even be in the form of droplets if all of the solvent has evaporated to leave only particulate (dry) matter. In the flame, atomisation and ionisation occur to give ions of the elements present (see the Back-to-Basics guide, *Plasma Torches*). The efficiencies with which solutions are transported from the inlet,

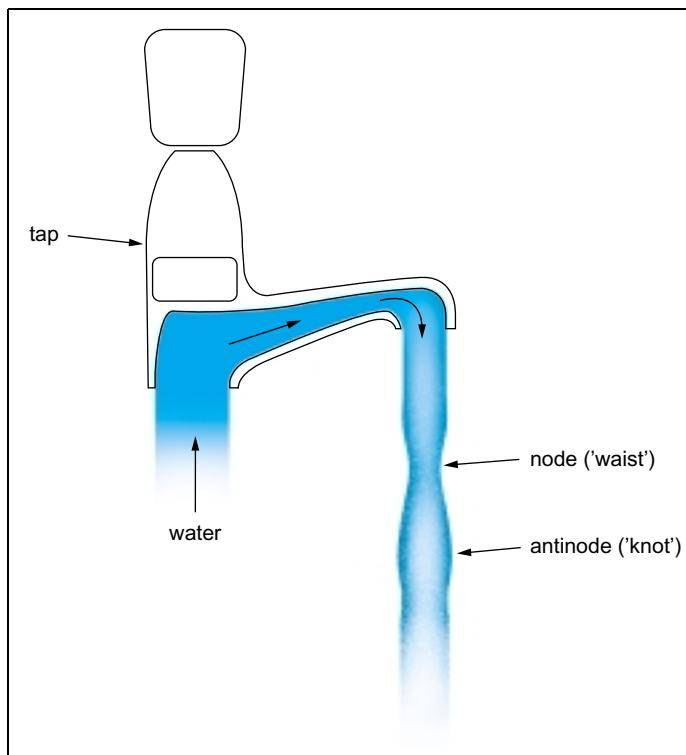


Figure 3. Water flowing from a tap begins as a steady, parallel-sided stream but this stream on flowing through the air begins to develop "waists" and "knots" as the flow becomes uneven. Together with associated turbulent flow, this leads to the stream breaking up completely, first into sections and then into large drops. This effect is best seen with a long narrow water fall. The water flows in an unbroken stream initially but, by the time it has accelerated and reached the end of its fall, it has broken apart. Where it hits the ground, a spray of smaller droplets is produced, exactly similar to the fine spray produced by an "impactor bead" placed in the path of an aerosol generated by a nebulizer.

through the nebulizer and along to the flame vary widely with the design of the nebulizer and frequently range from about 10 to 20%. A few nebulizers are capable of much higher transport efficiencies.

The solution to be nebulized may be a one-off sample, pumped or drawn into the nebulizer at a rate varying from a few microlitres per minute to several millilitres per minute. Alternatively, the supply of solution may be continuous, as when the nebulizer is placed on the end of a liquid chromatographic column.

There are many designs of nebulizer commonly used in ICP-MS but their construction and mode of operation may be collated into a small number of groups, viz., pneumatic, ultrasonic, thermospray, APcl and electrospray. These different types are discussed in the following sections, which are followed by further sections on spray and desolvation chambers.

Pneumatic nebulizers (PN)

Principles of operation

If a gas flows over the surface of a liquid, certain effects ensue. Only the *relative velocity* of the liquid surface and the gas is important in giving rise to nebulization. Thus, some pneumatic nebulizers work on the principle of using a high velocity gas stream (usually argon) passing over a liquid surface or through a liquid to produce an aerosol but others produce a fast moving stream of liquid, which is passed into an almost "stationary" gas. In either case, the relative velocity of the liquid and gas is the most important factor in production of an aerosol.

The early stages of pneumatic aerosol formation are familiar in everyday life. As an aeroplane flies, the flow of air over its wings causes a reduction in pressure just above the wing. The same principle is evident in sailing, in which boats are able to sail against the wind by making use of the reduction in pressure caused by a gas flow over a surface except that, for the boat the "wing" (sail) is vertical instead of horizontal. Water flowing from a tap in a steady stream is found to lose its even flow and to form "waists" and "knots" (Figure 3) as turbulence begins. In pneumatic nebulizers, the relative velocity of gas and liquid first induces a reduction in pressure above the surface of the liquid (see the calculation in Figure 4). The reduction in pressure is sufficient to cause liquids to flow out of capillary tubes, in accord with Poiseuille's formula (Figure 5). As the relative velocity of a liquid

Reduced pressure caused by a flowing stream

When a gas or liquid flows over a surface, the pressure at the surface is reduced according to the formula shown in equation (1), in which d is the density and v is the linear flow velocity of the moving stream.

$$\Delta p = d.v^2/2 \quad (1)$$

If d is measured in g.cm^{-3} and v in cm.s^{-1} then Δp is in dyn.cm^{-2} . This result can be converted approximately into atmospheres on multiplication by 10^{-6} .

Consider a flow of argon of 0.5 L/min through an annular space of $4 \times 10^{-4} \text{ cm}^2$ between two concentric capillary tubes at normal ambient temperatures. If the density of argon is taken to be $1.2 \times 10^{-3} \text{ g.cm}^{-3}$ then,

$$\text{Flow of argon} = 0.5 \text{ L/min} = 500 \text{ cm}^3/\text{min} = 8.7 \text{ cm}^3.\text{s}^{-1}$$

$$\text{Linear flow} = 8.7 / (4 \times 10^{-4}) \text{ cm.s}^{-1} = 2.2 \times 10^4 \text{ cm.s}^{-1}$$

$$\Delta p = d.v^2/2 = 1.2 \times 10^{-3} \times 4.8 \times 10^8 / 2 = 2.9 \times 10^5 \text{ dyn.cm}^{-2}$$

$$= 0.3 \text{ atmospheres}$$

Figure 4. The drop in pressure when a stream of gas or liquid flows over a surface may be estimated from the above approximate formula if viscosity effects are ignored. The example calculation reveals that, with the sorts of gas flows common in a concentric tube nebulizers, the liquid (the sample solution) at the end of the innermost tube is subjected to a partial vacuum of about 0.3 atmospheres. This vacuum causes the liquid to “lift” out of the capillary, where it meets the flowing gas stream and is broken up into an aerosol. For cross flow nebulizers, the vacuum created depends critically on the alignment of the gas and liquid flows but, as a maximum, it can be estimated from the above formula.

and a gas increases and, particularly if the mass of liquid is relatively small, this partial vacuum and rapid flow causes the surface of the liquid to be broken down into droplets. An aerosol is formed.

The sizes of the droplets formed in an aerosol has been examined for a range of conditions important in ICP-MS and can be predicted from an experimentally determined empirical formula (Figure 6). Of the two terms in the formula, the first is most important, except at very low relative flow rates. At low relative velocity of liquid and gas, simple droplet formation is observed but, as the relative velocity increases, the stream of liquid begins to “flutter” and to break apart into long thinner streamlets, which then break down into droplets. At even higher relative velocity, the liquid surface is stripped off and the thin films so formed are broken down into droplets. Thus, droplet formation occurs over a wide range of relative velocities of liquid and gas so that aerosols (droplets plus vapour) may be formed under a wide range of conditions. For ICP-MS purposes, it is preferable to have aerosols with very small droplets covering a narrow distribution of diameters. The various pneumatic nebulizers are constructed on the basis of these principles.

Concentric tubular nebulizers

Figure 7 shows a typical construction of a concentric tube nebulizer. The sample (analyte) solution is placed in the innermost of two concentric capillary tubes and a flow of argon is forced down the annular space between the two tubes. As it emerges, the fast flowing gas stream causes a partial vacuum at the end of the inner tube (Figure 4) and the sample solution “lifts” out (Figure 5). Where the emerging solution meets the fast flowing gas, it is broken down into an aerosol (Figure 7), which is swept along with the gas and eventually reaches the plasma flame. Uptake of sample solution is commonly a few mL per minute.

The dimensions of concentric tube nebulizers have been reduced to give microconcentric nebulizers (MCN), which may also be made from acid resistant material. Sample uptake with these microbore sprayers are only about 50 µL per minute and yet they provide such good sample transfer efficiencies that they have a performance comparable to other pneumatic nebulizers, which consume about 1mL per minute of sample.

Careful alignment of the ends of the concentric capillary tubes (the nozzle) and structural stability is essential. Commercially, three main types of arrangement are available A, C or K (Figure 8). The actual gas flow needed to produce a fine spray depends mainly on the nozzle geometry and the diameters of the two concentric tubes. Generally,

Poiseuille's formula

This predicts the rate of flow of liquid through a smooth tube under the effect of a pressure difference between the ends of the tube (equation 1).

$$F = (\pi \times r^4 \times \Delta p) / (8 \times \eta \times l) \quad (1)$$

In the equation, F is the flow of liquid ($\text{cm}^3.\text{s}^{-1}$) of viscosity η (poise or dyn.s.cm^{-2}) under a pressure drop (Δp dyn.cm^{-2}) through a tube (inner radius, r cm) of length l (cm).

From the example of Figure 1b, a flow of argon can cause a pressure drop of 0.3 atmospheres ($3 \times 10^5 \text{ dyn.cm}^{-2}$). Let the viscosity of the sample solution be the same as that of water 0.01 poise), the radius be 0.01 cm and the length of capillary be 10 cm.

$$F = (\pi \times 0.01^4 \times 3 \times 10^5) / (8 \times 0.01 \times 1) = 1.2 \times 10^{-2} \text{ cm}^3.\text{s}^{-1} (0.7 \text{ mL/min})$$

Under these conditions, the flow of sample liquid is predicted to be shown and this is similar to the flows observed with concentric nebulizers.

Figure 5. Using Poiseuille's formula, the calculation shows that for concentric tube nebulizers, with dimensions similar to those in use for ICP-MS, the reduced pressure arising from the relative linear velocity of gas and liquid causes the sample solution to be pulled from the end of the inner capillary tube. It can be estimated that the rate at which sample is through the inner capillary will be about 0.7 mL/min. For cross flow nebulizers, the flows are similar, once the gas and liquid stream intersection has been optimised.

gas flows of about 1L per minute lead to sample solution flows of about 0.5 - 4 mL per minute. A special design of a high efficiency nebulizer (HEN) uses much narrower capillary tubes, which are about 100 µm in diameter. These afford sample flows of about 10 µL per minute and give a narrow distribution of droplet sizes, of mean diameter close to 10 µm. This small droplet size means that transfer efficiency of the sample into the plasma flame is high. To attain the required gas flow for nebulization of analyte solution at a rate of about 1L per minute, a much higher argon gas operating pressure is needed. One problem with the very narrow bore tubes is that they can block easily due to small pieces of particulate matter in the liquid solution sticking inside them. Some form of prefilter is preferable.

In a different arrangement, the relative velocity of liquid and gas is achieved differently. Now, the sample is pumped at high pressure (6000 psi) through a fine capillary tube (30 µm diameter) so that it emerges as a very fast moving stream, which meets argon gas. The latter is arranged to flow relatively slowly to the plasma flame. Where the fast moving liquid meets the slow moving gas, aerosol formation occurs as described above and this is transported to the plasma flame.

In these and other pneumatic nebulizers, the first formed aerosol is sometimes broken down into even smaller droplets by simply allowing it to impinge onto a solid target. The mechanical effect of small droplets being flung against a solid object leads to further fragmentation of the droplets (Figure 9). The target is usually a glass bead, this shape being chosen so as to encourage suitable gas flow around it. The target is known as an "impactor bead". One problem with the very fine capillaries used in these and other such devices is the possibility of blockages occurring through particulate matter suspended in the sample solutions. With some solution types, the ends of the capillaries may become blocked by deposition of solids from the evaporating solution.

In an even simpler arrangement of the concentric tube concept, the liquid and fast flowing argon gas streams are simply mixed before they issue from the nebulizer. For this to work well, the solution needs to be pumped into the nozzle area. A higher gas pressure is needed to give a good nebulization. This device (Figure 10) gives a very finely dispersed spray and provides a good sample transfer rate.

$$D = \frac{585}{V} \sqrt{\frac{\sigma}{d}} + 597 \left(\frac{\gamma}{\sqrt{d \cdot \sigma}} \right)^{0.45} \times (1000Q)^{1.5}$$

D = mean droplet diameter (m)

σ = surface tension (dyne. cm⁻¹)

d = density of liquid (g. cm⁻³)

γ = viscosity of liquid (poises)

V = difference in linear velocities of gas and liquid flows (m/s)

Q = ratio of volume flows of liquid / gas

For water solutions, the following approximate values can be used:

$$\sigma = 73; d = 1; \gamma = 0.01; Q = 10^{-3} \text{ (1 L.min}^{-1} / 1 \text{ L.min}^{-1}\text{)}$$

For $V = 50$ m/s, $D \approx 120$ m

Note that the formula is valid only for $30 < \sigma < 73$; $0.01 < \gamma < 0.3$; $0.8 < d < 1.2$; $5 < V < 50$

Figure 6. The above formula for estimating droplet size was determined experimentally. Of the various terms, the first is the most important for small values of V . As V becomes small, the second term gains in importance. Unless the density or viscosity of the sample solution changes markedly from the values for water, mean droplet size can be estimated approximately by using the corresponding values for water, as shown.

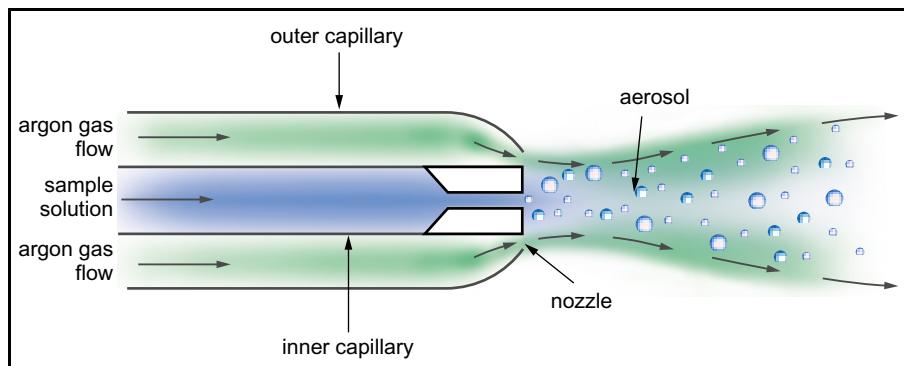


Figure 7. In a concentric tube nebulizer, the sample solution is drawn through the inner capillary by the vacuum created when the argon gas stream flows over the end (nozzle) at high linear velocity. As the solution is drawn out, the edges of the liquid forming a film over the end of the inner capillary are “blown” away as a spray of droplets and solvent vapour. This aerosol may pass through spray and desolvation chambers before reaching the plasma flame.

Cross flow nebulizers

(a) Capillary tubes

The flows of gas and liquid do not need to be concentric for aerosol formation and, indeed, the two flows could meet at any angle. In the cross flow nebulizers, the flows of gas and sample solution are approximately at right angles to each other. In the simplest arrangement (Figure 11), a vertical capillary tube carries the sample solution. A stream of gas from a second capillary is blown across this vertical tube and creates a partial vacuum so that some sample solution lifts out onto the top of the capillary. There, the fast flowing gas stream breaks down the thin film of sample solution to form an aerosol, which is swept on to the plasma flame. Again, an impactor bead may be used to produce greater breakdown into fine droplets. The cross flow arrangement is less prone to blocking from build-up of solids from the evaporating analyte. For best performance, the capillaries need to be placed very carefully into an optimum relationship to each other and, for this reason, may need to be adjustable. Such an arrangement may lead to the capillaries oscillating in the gas stream and, to obviate this effect, one design (MAK) uses thick-walled, small diameter, rigid capillaries and a high argon gas pressure of about 200 psi to achieve an argon gas flow of about 0.5 L per minute. Like other cross flow devices, this last is particularly free from clogging and gives good long-term stability.

(b) Liquid films

This form of cross flow nebulizer produces excellent aerosol sprays. In general, with these devices, a thin film of the sample solution attempts to cover a small orifice through which argon gas flows, at right angles to the putative film. The thin film of liquid around the rim of the orifice is blown apart to form an aerosol (Figure 12). The sample solution may flow under gravity onto the orifice or may be pumped there. This apparatus for forming an aerosol is frequently called a "Babington" nebulizer. The cross-flow again provides freedom from clogging and can even be used with slurries and not just solutions. The efficiency in aerosol formation with these devices arises from the thin line of liquid surrounding the orifice, which allows the fast flowing gas stream to break it up into a fine spray. With this simplest of arrangements, much of the sample solution does not reach the orifice and flows away to a drain, from which it can be recycled. This is a complicated procedure and other ways have been found for providing a thin film and a gross flow of gas.

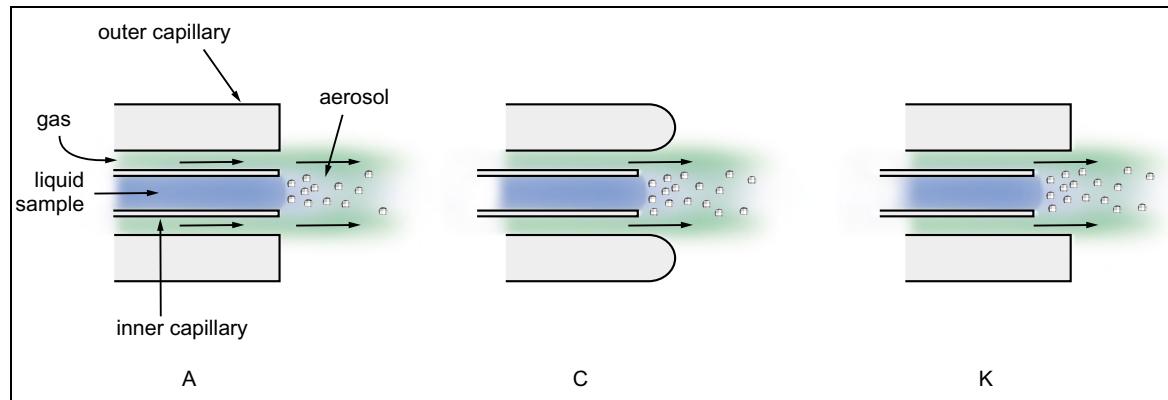


Figure 8 Three common types of nozzle are shown diagrammatically. Types A and K are similar, with sharp cut-offs on the ends of the outer and inner capillaries so as to provide better shear forces on the liquid issuing from the end of the inner tube. In types K and C, the inner capillary does not extend to the end of the outer tube and there is a greater production of aerosol per unit time. These concentric tube nebulizers operate at argon gas flows of about 1L/min.

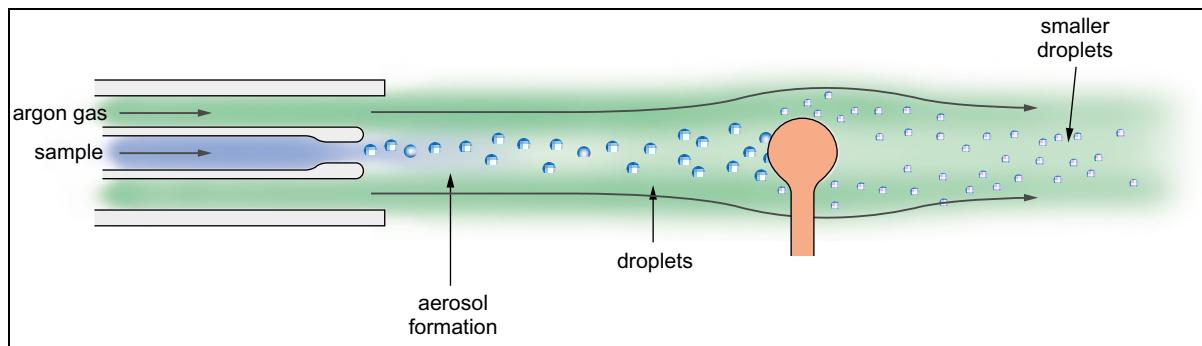


Figure 9 The fast flowing narrow liquid stream has a high relative linear velocity with respect to the slower flowing argon gas stream. This leads to the liquid stream being broken up into fast moving droplets, which strike the impactor bead and form much smaller droplets.

In the so-called “V-groove” device, sample solution flows as a thin stream along a V-shaped groove cut into a metal block. A gas flow is arranged almost coincident with and in the direction of the groove. The fast flowing gas passing over the liquid stream produces a fine aerosol. Much more of the solution is transferred to the plasma flame than is the case for a simple Babington nebulizer. The V-groove may be incorporated into a capillary cross-flow nebulizer (Figure 13) and, in yet another arrangement, the liquid film flows from a V-groove and onto a platinum grid (fine wire mesh), where the liquid presents a high surface area as it covers the wires. Fast flowing argon gas flows through the grid, producing an aerosol. For even better droplet dispersion and small size, there is normally a second platinum grid placed behind the first. This second grid acts in the same way as an impactor bead, breaking up small drops into even smaller ones (Figure 14).

Another variant (the “Cone spray”), allows the sample solution to flow down the sides of an inverted cone, through a hole in the bottom of which flows a fast stream of argon gas. As the liquid film meets the gas, it is ripped apart into a finely dispersed aerosol (Figure 15).

Finally, in yet another variant, the sample liquid stream and the gas flow are brought together at a shaped nozzle into which the liquid flows (parallel path nebulizer). Again, the cross intersection of liquid film and gas flow leads to the formation of an aerosol. Obstruction of the sample flow by formation of deposits is not a problem and the devices are easily constructed from plastics, making it robust and cheap.

(c) Frit nebulizers

The aim of breaking up a thin film of liquid into an aerosol by a cross flow of gas has been developed with frits, which are essentially a means of supporting a film of liquid on a porous surface. As the liquid flows onto one surface of the frit (frequently made from glass), argon gas is forced through from the undersurface (Figure 16). Where the gas meets the liquid film, the latter is dispersed into an aerosol and is carried as usual towards the plasma flame. There have been several designs of frit nebulizers but all work in a similar way. Mean droplet diameters are approximately 100 nm and over 90% of the liquid sample can be transported to the flame. There are problems in use of the frit nebulizer. Memory effects tend to be severe and each sample needs to be followed by several “wash-outs” with clean solvent before the pores of the frit become free of residual sample. Biological samples frequently contain detergent-like materials and these give

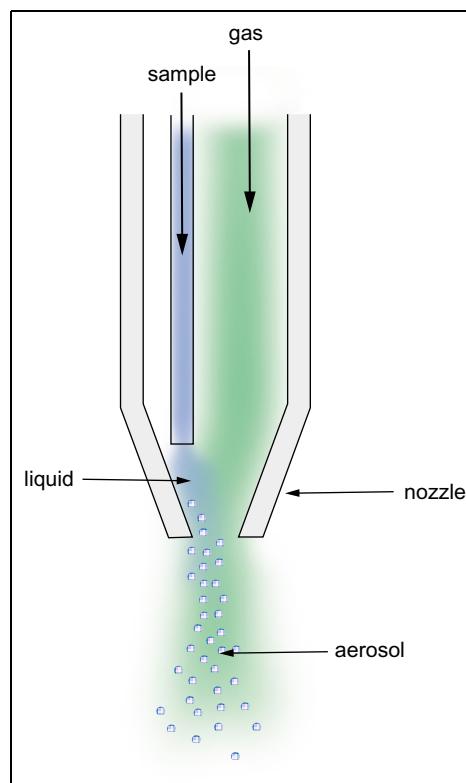


Figure 10 The liquid sample flows into the nozzle and coats the inside walls. The sample stream arrives at the orifice (the nozzle outlet is about 0.01 cm diameter), where it meets the argon stream and is nebulized.

rise to frothing on the frit when gas is blown through them. However, in suitable instances, the frits perform well, especially with small volumes of sample, which may be discrete or continuous.

Ultrasonic nebulizers (USN)

Principle of operation

If ultrasound of a frequency in the range approximately 0.2–10 MHz is arranged to pass through a liquid then, at the surface of the liquid, longitudinal rarefaction and compression waves break up the surface. If the amplitude of the waves is sufficient, *viz.*, if there is sufficient power then the surface of the liquid is disrupted to form a stream of droplets (an aerosol). This is the basis of the ultrasonic nebulizer. The ultrasound is usually produced piezo-electrically.

For any ultrasound of frequency ν , the longitudinal wavelength at the surface can be calculated from equation (1), in which σ is the surface tension of the liquid and ρ is the density of the liquid.

$$(1) \quad \lambda = (8\pi\sigma/\rho\nu^2)^{1/3}$$

For example, at a frequency of 1 MHz, the effect of ultrasound in water of surface tension 73 dyne.cm and density 1 g.cm⁻³, is to produce longitudinal waves of about 12 μm. The resulting mean droplet diameter (D) is given by equation (2).

$$(2) \quad D = 0.34\lambda$$

For a longitudinal disturbance of wavelength 12 μm, the droplets have a mean diameter of about 3–4 μm. These very fine droplets are ideal for ICP-MS and may be swept into the plasma flame by a flow of argon gas. Unlike pneumatic forms of nebulizer in which the relative velocities of the liquid and gas are most important in determining droplet size, the flow of gas in the ultrasonic nebulizer plays no part in the formation of the aerosol and serves merely as the droplet carrier.

(a) Piezoelectric transducer nebulizers

Application of an AC voltage of high frequency to a piezoelectric crystal causes faces of the crystal to move back and forth at the same frequency. This is a piezo transducer. If one of the piezo crystal faces is immersed in a liquid, the oscillatory motion transmits longitudinal ultrasound waves into the liquid. At the surface, the longitudinal waves disrupt it as compression and rarefaction waves arrive there.

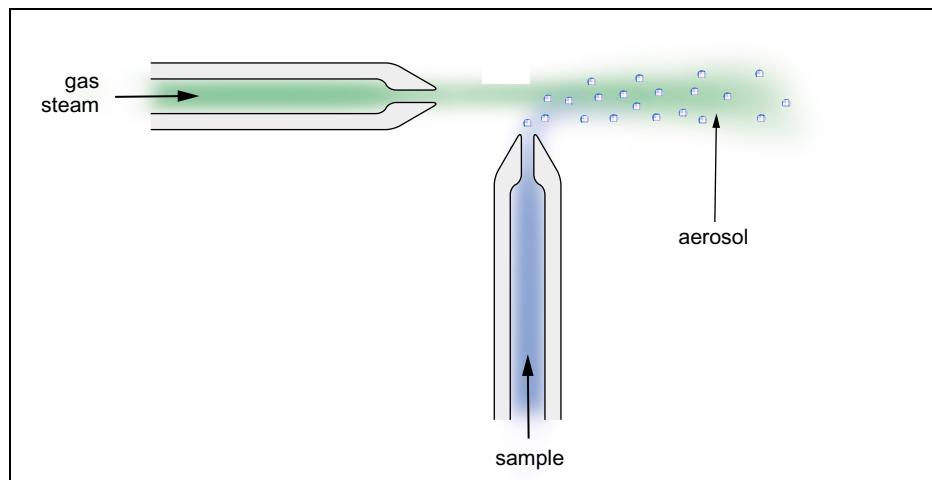


Figure 11 In the cross flow arrangement, the argon gas flows at high linear velocity across the face of an orthogonal capillary tube containing sample solution. The partial vacuum causes liquid to lift above the level of the end of the capillary. Here, it meets the argon and is nebulized.

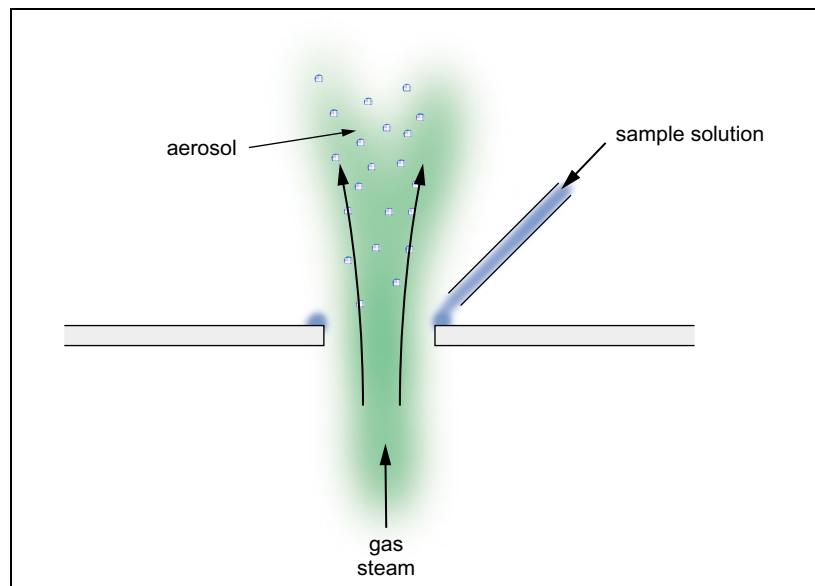


Figure 12 In this cross flow arrangement, a thin film of sample solution is obtained as it flows around the edge of a small opening, through which there is a fast linear flow of argon. The liquid film is rapidly nebulized along the rim of the orifice.

The break up of the surface leads to drops being formed and these spray away from the surface. The rate of formation of drops is similar to the frequency of disruption of the surface. Thus, a 100 KHz ultrasound wave produces about 10^5 droplets per second. This rate of formation of droplets is some thousands of times greater than their rates of formation in pneumatic nebulizers.

Therefore, with normal argon flows, much more material is carried towards the plasma flame per unit time than is the case with pneumatic devices. Even so, there may be a need for a desolvation chamber to remove as much solvent as possible before the droplets or particulates reach the flame, if the performance of the latter is not to be affected. The ultrasonic devices have greater need still for desolvation because of their higher rate of droplet formation and higher rate of transfer of sample solution. However, at the highest frequencies (1 MHz), droplet size is small and natural desolvation by evaporation is so rapid that a special desolvation chamber becomes unnecessary.

The transfer efficiencies for ultrasonic nebulizers (USN) are about 20% with a sample uptake of about 1 mL per minute. Almost 100% transfer efficiency can be attained at lower sample uptakes of about 5–20 μ L per minute. With ultrasonic nebulizers, carrier gas flows to the plasma flame can be lower than for pneumatic nebulizers because they transfer sample at a much higher rate. Thus, reduction in the carrier gas flow means that the sample remains in the mass measurement system for longer and this provides much better detection limits.

Ultrasonic nebulizers are almost free of clogging from solute, have better detection limits and have become popular despite their high cost relative to the pneumatic forms. A typical construction of an ultrasonic nebulizer is shown in Figure 17.

To accommodate smaller liquid flows of about 10 μ L per minute, microultrasonic nebulizers have been designed. Although basically similar in operation to standard ultrasonic nebulizers, in these micro varieties, the end of a very small diameter capillary, through which is pumped the sample solution, is in contact with the surface of the transducer. This arrangement produces a thin stream of solution, which runs down and across the centre of the face of the transducer. The stream of sample solution is broken down into small diameter droplets by standing waves in the analyte solution, which is transferred to the plasma flame with very high efficiencies.

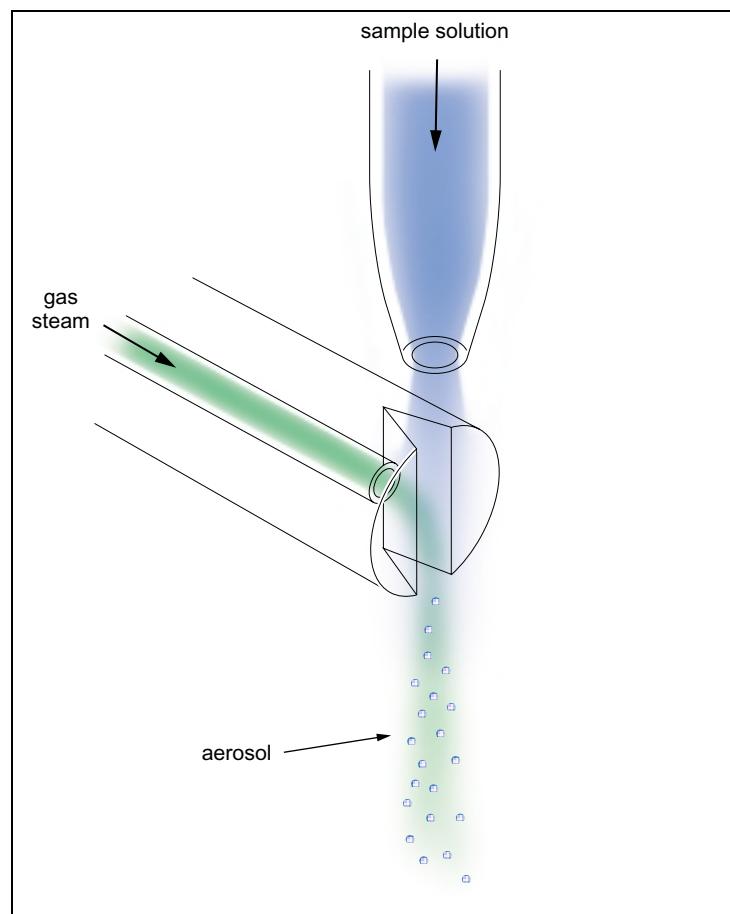


Figure 13. A sample solution is drawn or pumped into a V-shaped groove cut into the end of a capillary tube. The crossed gas and liquid streams form an aerosol. An impactor bead may be used to provide an even smaller droplet size.

Oscillating capillary

In appearance, this device resembles the concentric tube capillary pneumatic nebulizers but it operates on a different principle. As with the pneumatic type described above, the sample solution passes through the inner capillary (diameter about 50 µm) and argon gas is blown through the annular space between the inner and outer capillaries (diameter about 250 µm). Unlike the pneumatic concentric tube nebulizers, the inner capillary tube is not held rigidly but is allowed to vibrate as gas flows past the end of it. This transverse or “fluttering” motion may be likened to the fluttering of a flag in a strong wind. The solution stream leaving the end of the inner capillary is subjected to this transverse vibration and begins to break up into segments. At the same time as the transverse wave is being produced mechanically, an acoustic wave is generated in the liquid (again, this may be compared with the noise made by a fluttering flag). The acoustic wave travels longitudinally along the liquid stream, whilst the latter is undergoing transverse mechanical vibrations. At a frequency of about 1 KHz, this longitudinal wave has a wavelength of about 15 µm and leads to the stream being broken down into smaller droplets (equation 2; see earlier). This type of nebulizer delivers good performance over a range of sample solution flows (1 µL to 1 mL per minute) and with a range of solvents. Accordingly, the oscillating capillary nebulizer provides a convenient interface between a liquid chromatographic apparatus and a plasma flame.

**Thermospray
nebulizers
(TN)****Principle of operation**

In one sense, the thermospray nebulizer could be considered as a pneumatic device, in which a fast-flowing argon gas stream is replaced by a very rapidly vaporising flow of solvent from the sample solution itself. A typical arrangement of a thermospray device is shown in Figure 18.

The sample solution is pumped along a narrow capillary tube, the end of which becomes the nozzle of the nebulizer. On the outside of the capillary near its nozzle end, an electrical heater rapidly warms the thin stream of liquid inside the capillary so that the solvent vaporises very quickly. The fast expanding vapour mixes with unvaporised solution and blows it out of the end of the capillary as an aerosol. An argon carrier gas is used to ferry the aerosol to the plasma flame; the gas generally plays little or no role in formation of the droplet spray.

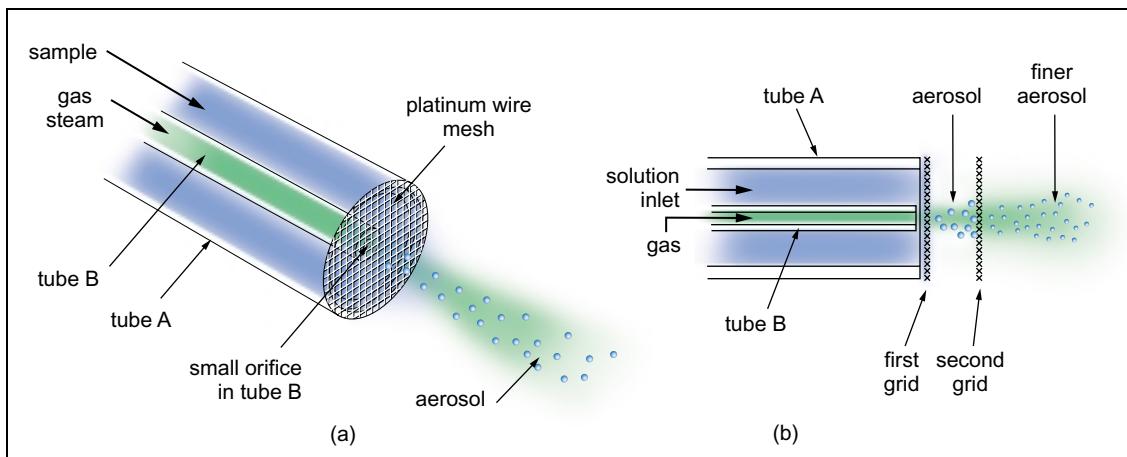
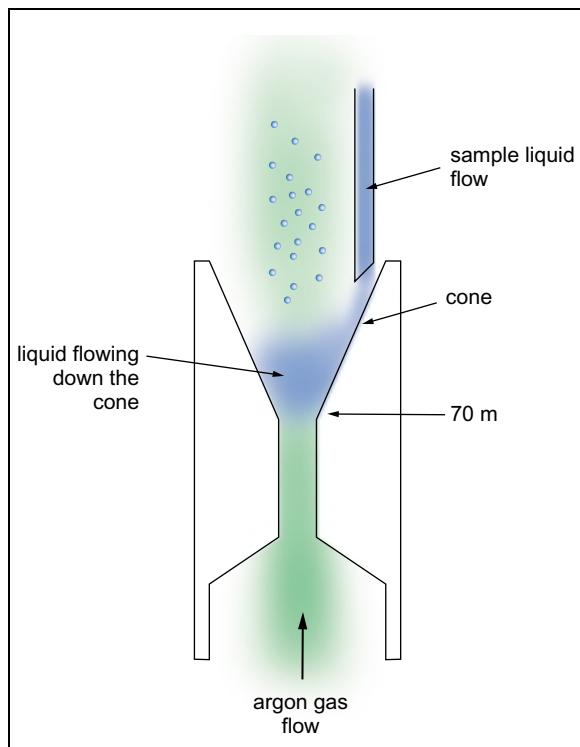


Figure 14. (a) The sample solution flows to the end of a tube A, where it enters a groove cut into the end of the tube. The liquid runs as a thin film down the grid wires of a fine platinum mesh. Argon flows through the inner tube B and issues from a small orifice placed just in front of the mesh. The high linear velocity of the argon passing through the mesh and over the thin film of liquid. (b) To aid further reduction in droplet size, a second grid is placed after the first and is used as an impactor (cf. impactor beads).

Figure 15. Again, this arrangement provides a thin film of liquid sample solution flowing down to a narrow orifice (0.007 cm diameter) through which argon flows at high linear velocity (volume flow is about 0.5-l L/min). A fine aerosol is produced. This particular nebulizer is efficient for solutions having a high concentration of analyte constituents



As the droplets are already warm, further evaporation of solvent is rapid. More discussion of this process may be found in the Back-to-Basics guide, *Thermospray and Plasmaspray*.

The thermospray device produces a wide dispersion of droplet sizes and transfers a lot of sample solution in unit time to the plasma flame. Therefore, it becomes essential to remove as great a proportion of the bigger droplets and solvent as possible if flame performance is not to be compromised and, for these reasons, both spray and desolvation chambers are needed. This is need is especially so for analyte solutions in organic solvents.

Thermospray nebulizers are somewhat expensive but can be used on-line to a liquid chromatographic column. About 10% of sample solution is transferred to the plasma flame. The overall performance of the thermospray device compares well with pneumatic and ultrasonic sprays. When used with microbore liquid chromatographic columns, which produce only about 100 µL per minute of eluant, the need for spray and desolvation chambers is reduced and similar detection sensitivities to those of the ultrasonic devices can be attained, both being some 20 times better than the sensitivities routinely found in pneumatic nebulizers.

Electrospray nebulization (EN)

For a discussion of droplet and ion formation in electrospray mass spectrometry, please see the Back-to-Basics guide, *Electrospray*.

Electrospray nebulizers were used for the formation of ionic aerosols before they were used as general inlets in organic chemical applications, particularly in conjunction with liquid chromatography. Two effects may operate, one electrical, the other pneumatic (Figure 19).

The sample solution flows or is pumped along a capillary tube, the end of which is held at a high positive or negative electrical potential. Because of the electrical charge, the surface of the solution at the outlet of the capillary also becomes charged and is repelled by the existing electric field of the same sign. If the capillary tube is narrow enough, the liquid inside is forced out of the end of the capillary and the surface of the liquid is rounded with a high radius of curvature. This “point” of liquid leads to a steady stream of charged droplets being repelled into a desolvation chamber. If the charged capillary tube is also surrounded by an uncharged concentric capillary, argon or other gas can be blown through the annular space between the capillaries and this can be used to aid droplet formation, as with concentric capillary pneumatic nebulizers. The electric field and gas flow work together to provide a finely dispersed spray of charged

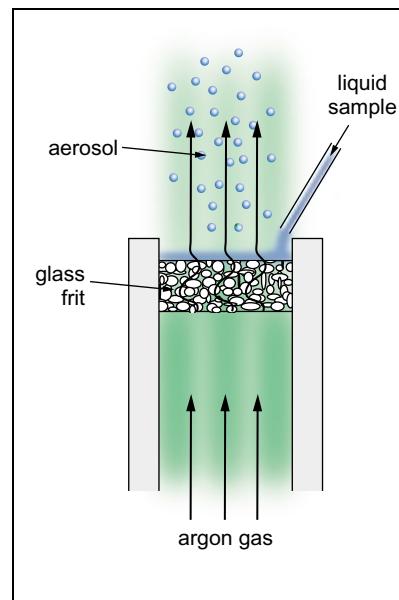


Figure 16. The sample solution flows onto a piece of fritted glass, through which argon gas flows. The flow of argon is broken down into narrow parallel streams of high linear velocity, which meet the thin film of liquid percolating into the pores of the frit. At the interfaces, an aerosol is formed and is blown off from the top of the frit.

droplets. An electric potential of about 3–5 kV is used for capillaries of about 50–100 µm diameter. Even without a rapid gas flow, the droplets produced are very small at about 1 µm diameter or less and they are produced at a rate of about 10^8 per second, similar to the rate of production of droplets in an ultrasonic device.

For use in ICP-MS, the charged droplets produced by the electrospray nebulizer pose a problem. The droplets do not coalesce because they carry the same sign of charge, viz., they are either all positively or all negatively charged depending on the sign of the applied electric potential. This same charge leads to the droplets repelling each other and spreading the aerosol spray; many droplets will be attracted to the opposite electrode or to ground potential, causing them to migrate to the walls of the nebulizer or desolvation chamber. Additional to loss of material, the charged species provide an electrically conducting path in an otherwise non-conducting gaseous medium. As described in Back-to-Basics, *Coronas, Plasmas and Arcs*, this is a recipe for an electrical discharge. An electrical discharge may interfere with the sensitive detection electronics of the instrument so as to produce a “spike” in the measurement of m/z values. It is essential to remove the charges from the droplets before they can be used in ICP-MS and this has been done by adding air or water vapour to the argon carrier gas. Removal of charge from the droplets is achieved by their reaction with oxygen or water (Figure 20). Although this scheme reduces the problem of electrical discharges, it does not remove the problem entirely.

Spray and desolvation chambers

These adjuncts to nebulizers have been mentioned above. The aerosol produced by a nebulizer consists of solvent vapour and droplets of solution, with sometimes even small pieces of particulate matter. The solvent diffuses into the argon carrier gas and the droplets are swept along by the gas. Before reaching the plasma flame, it is necessary to remove as much solvent vapour as possible so as not to upset its performance. Large droplets in the aerosol do not have time to desolvate by natural evaporation before reaching the flame and these relatively large amounts of material entering the flame can cause serious instabilities. Therefore, as well as nebulizing a solution, it may be necessary to modify the aerosol before it reaches the plasma flame. Some or even all of this modification may be avoided by good design of the nebulizer but, in the cases when such modification is needed, it normally takes one or both of two forms.

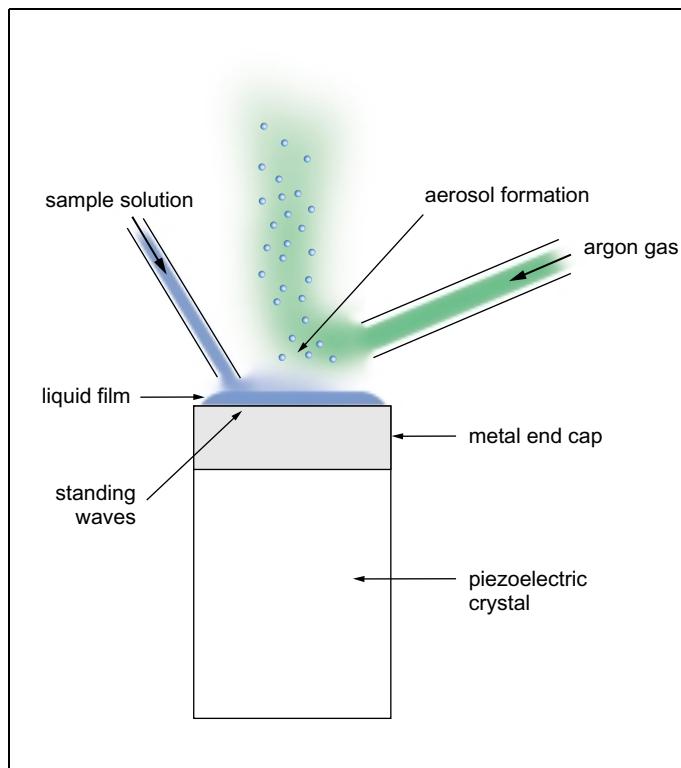


Figure 17. In this graphical representation of an ultrasonic nebulizer, the sample solution spreads as a thin film across the face of a piezoelectric transducer. Standing longitudinal waves produce a spray of very small droplets, which are transported to the plasma flame by a flow of argon gas. The argon plays no part in manufacturing the aerosol and is used merely as a carrier gas flowing at 0.5-1 L/min.

The first form of aerosol modifier is a spray chamber. This is designed to produce turbulent flow in the argon carrier gas and to give time for the larger droplets to coalesce by collision. The end result of coalescence, gravity and turbulence is to deposit the larger droplets onto the walls of the spray chamber, from where the deposited liquid drains away. Since this liquid is all analyte solution, clearly some sample is wasted so that, when sensitivity of analysis is an issue, it may be necessary to recycle this drained off liquid back through the nebulizer.

Having removed the larger droplets, it may remain only to encourage natural evaporation of solvent from the remaining small droplets by use of a desolvation chamber. In this, the droplets are heated to temperatures up to about 150 °C, often through use of infrared heaters. The extra heat causes rapid desolvation of the droplets, which frequently dry out completely to leave the analyte as small particles that are swept by the argon flow into the flame.

Having assisted desolvation in this way, the carrier gas then carries solvent vapour produced in the initial nebulization with more produced in the desolvation chamber. The relatively large amounts of solvent may be too much for the plasma flame, causing instability in its performance and, sometimes, putting out the flame completely. Therefore, the desolvation chamber usually contains a second section placed after the heating section. In this second part of the desolvation chamber, the carrier gas and entrained vapour are strongly cooled to temperatures of about 0 to –10 °C. Much of the vapour condenses out onto the walls of the cooled section and is allowed to drain away. Since this drainage consists only of solvent and not analyte solution, it is normally directed to waste.

Introduction of sample solution via a nebulizer may need both spray and a desolvation chambers but a well-designed efficient nebulizer needs neither.

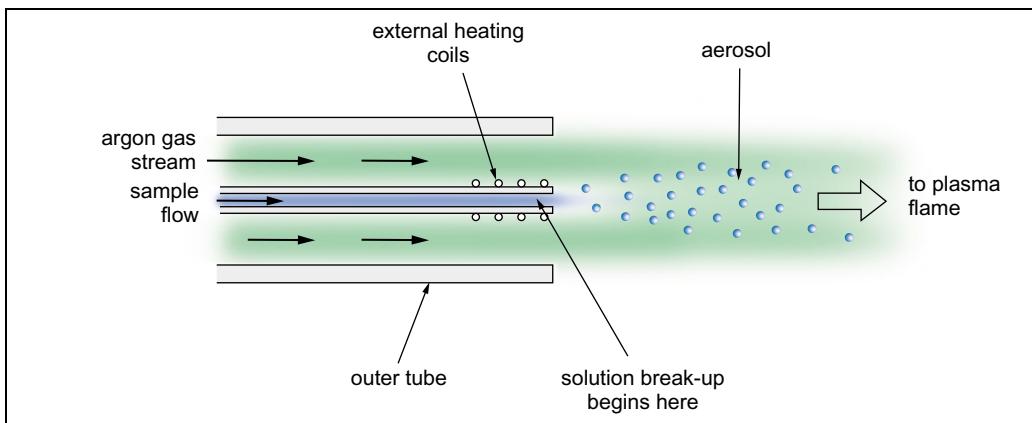


Figure 18. The sample solution is pumped (e.g., from the end of a liquid chromatographic column) through a capillary tube, near the end of which it is heated strongly. Over a short of tube, some of the solvent is vaporised and expands rapidly. The remaining liquid and the expanding vapour mix and spray out of the end of the tube as an aerosol. A flow of argon carries the aerosol into the plasma flame.

Conclusion Nebulizers are used for introducing analyte solutions as an aerosol spray into a mass spectrometer. For use with plasma torches, it is necessary to produce a fine spray and to remove as much solvent as possible before the aerosol reaches the flame of the torch. Various designs of nebulizer are available but most work on the principle of interacting gas and liquid streams or the use of ultrasonic devices to cause droplet formation. For use of nebulization in thermospray, APcl and electrospray, the relevant Back-to-Basics guides should be consulted.

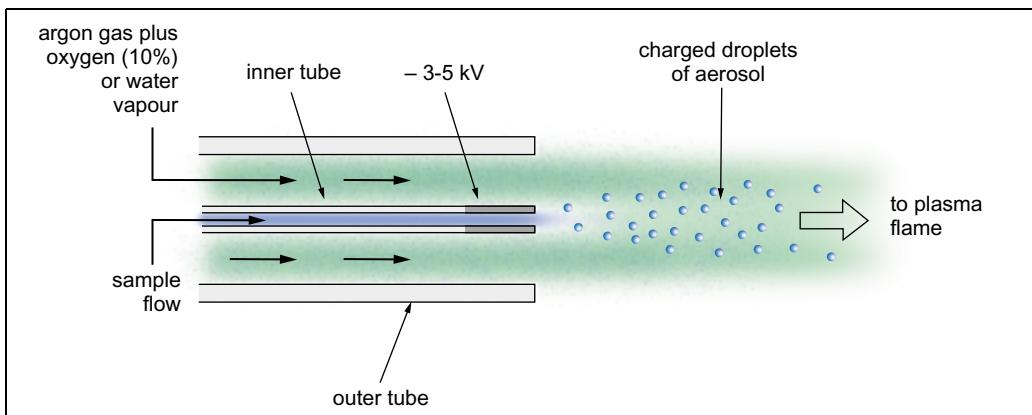


Figure 19. At the high potential on the end of the inner capillary tube, the analyte solution is forced out of the end of the tube and sprays into the argon stream as a charged aerosol. Droplet formation may be assisted by arranging the gas flow through a concentric outer tube (uncharged), similar to a concentric tube nebulizer. Oxygen or water vapour are added to the argon stream to remove electrical charges from the droplets.

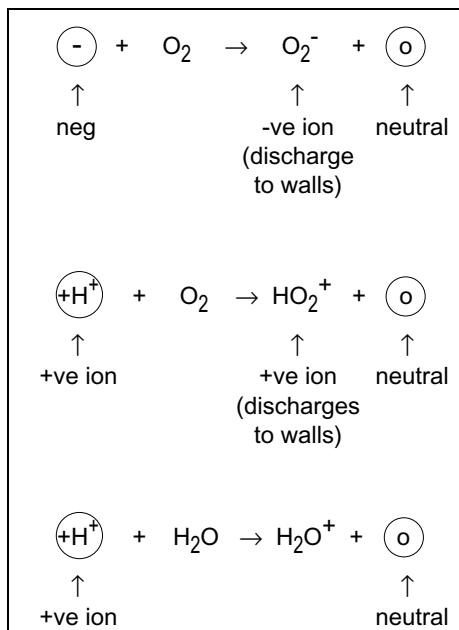


Figure 20. During “charge neutralisation”, oxygen or water molecules accept electrons or protons or other charges atoms from the droplets. Having lost their electrical charges and becoming neutral, the droplets remain in the main gas stream and are carried into the plasma flame. The small charged species formed by these exchange reactions migrate rapidly from the main gas stream and are discharged at the walls of the containment vessel.

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CHAPTER C2

HYBRID ORTHOGONAL-TOF INSTRUMENTS

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Quick Guide

- Time-of-Flight (TOF) instruments utilize the times taken by ions to pass (fly) along an evacuated tube as a means of measuring m/z values and therefore of obtaining a mass spectrum.
- TOF instruments may be operated as stand alone mass spectrometers (in-line with an ion source) but, frequently, they are used in combination with other techniques so as to give hybrid instruments. In such hybrids, the TOF analyser is usually placed at right angles (orthogonal) to a beam of ions and hence the term orthogonal TOF hybrids.
- Orthogonal TOF is the name commonly given to what should properly be called orthogonally accelerated Time-of-Flight mass spectrometry. Therefore, it is sometimes referred to by the acronym oaTOF, especially in official publications, but it is more usual to hear it referred to simply as orthogonal TOF, the abbreviation used in these notes.
- For stand alone or hybrid TOF mass spectrometry, the ions being examined must all start from some point at the same instant. From this zero time, the ions are accelerated through a short region having an electric potential of several kilovolts so as to achieve steady velocities, which vary in proportion to the square root of their m/z values.
- The process is rather like a sprint race, all ions leaving the 'starting line' at the same time. However, unlike a normal race, the result is always the same, viz., the ions arrive at the finish (collector or detector) in procession and strictly in the order of increasing m/z values. Ions of the smallest m/z values arrive first, followed successively by others of increasing m/z value.
- Flight times are extremely short (microseconds) for all of the ions and therefore the 'scanning' of the total mass spectrum from m/z 1 to about m/z 2,000-3,000 appears to be instantaneous on a human time scale. The arrival of ions at the finishing point is determined by a microchannel plate collector (detector).
- TOF instruments have been hybridized with sector and quadrupole analysers and with liquid chromatographs. These arrangements produce decided advantages over each technique alone.

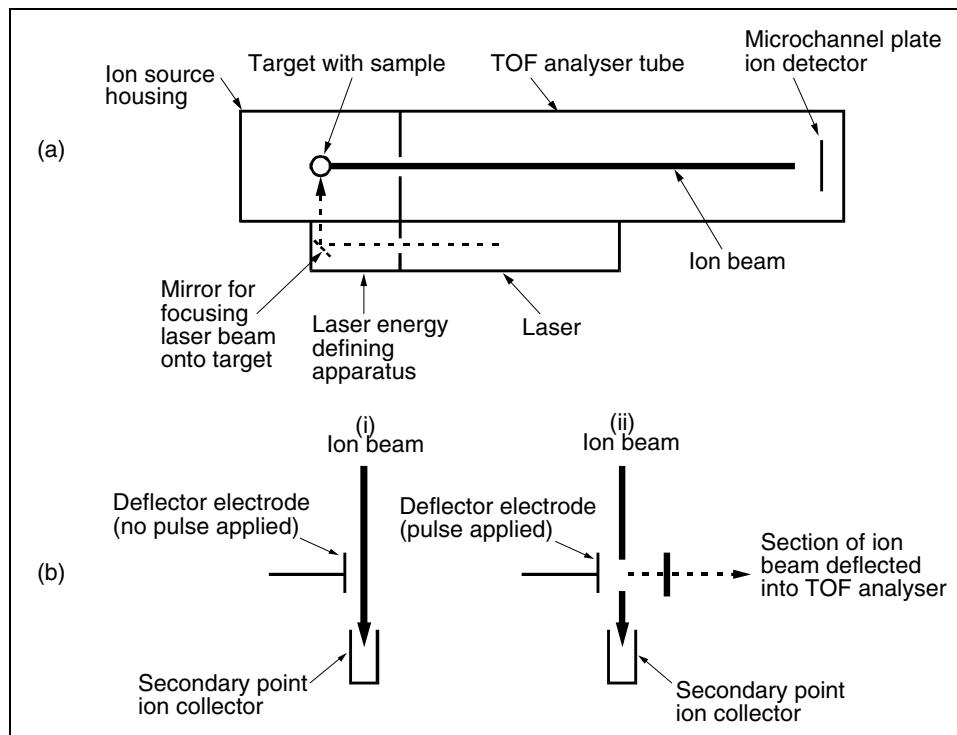


Figure 1 In (a), the outline of a simple in-line TOF mass spectrometer is shown. Ions produced by a 4 ns pulse of laser light onto the sample produces ions, which are accelerated through about 25 kV and directed into the flight tube. The times taken for the ions to reach a microchannel plate collector and the number arriving are recorded and these times are then converted into m/z values and abundances so as to obtain a mass spectrum. After several microseconds, the laser may again be fired and another spectrum obtained. The process may be continued as long as there is sample.

In (b), a continuous ion beam produced in one source may be detected by a point ion collector placed in line with the beam; this detector is usually a secondary collector. There is no electric field applied to the deflector electrode in (i). However, in (ii), an electric field of 1-5 kV has been applied to the electrode for about 10 ns. This detaches a short length of the main ion beam, which is accelerated almost at right angles to its original direction and proceeds into a TOF analyser. This last analyser produces a full spectrum, scanned in a few μs . After the pulse, the main ion beam continues as it did before the pulse was applied. Pulses may be applied at a rate of several kHz.

- Because TOF spectra are obtained in such a short time frame, further spectra may be accumulated rapidly. Thus, in one second it is possible to accumulate several thousand mass spectra.
- This attribute of rapid accumulation of spectra leads to excellent reproducibility and better signal-to-noise characteristics, and makes full use of small quantities of sample.
- In the orthogonal mode, a small section of an ion beam is sampled into the TOF analyser by use of a pusher electrode. Thus, an electrode placed alongside a positive ion beam and carrying a pulsed positive electric potential will deflect sections of the beam away from the main beam and approximately at right angles to it.
- Advantages of hybrid TOF instruments vary with the actual hybrid and are discussed in the following relevant sections.

Summary Hybrid Time-of-Flight mass spectrometers make use of a Time-of-Flight (TOF) analyser placed at right angles to a main beam. Ions are deflected at right angles into the TOF analyser by a pulsed electrical potential from an electrode placed alongside the beam. Hybrid TOF instruments have many advantages arising from the combination of two techniques, either of which alone would not be so useful.

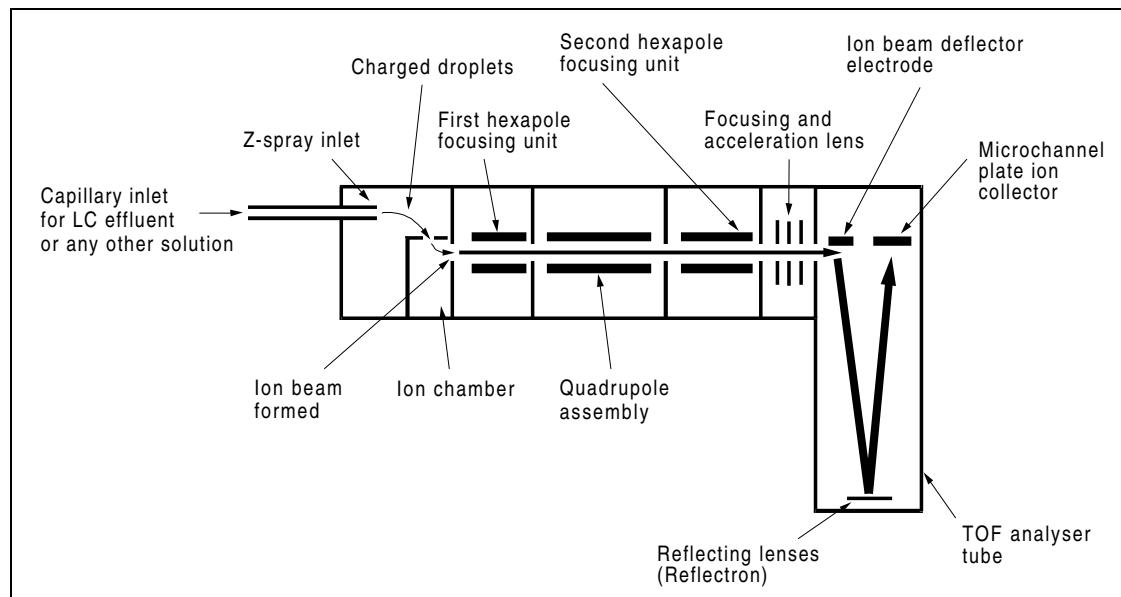


Figure 2 A solution containing the analyte of interest is sprayed from the end of a capillary by application of a high electric potential. The resulting charged droplets are stripped of solvent and ions formed from analyte molecules are directed electrically into the mass spectrometer (Z-spray). The ion beam passes into a quadrupole analyser, which may be operated in a narrow bandpass mode so as to transmit ions of defined m/z values or in its wide bandpass mode, in which all ions are transmitted irrespective of m/z value. There is a further focusing hexapole, after which the ion beam is focused and accelerated by an electric lens before being passed into the TOF analyser in front of a deflector electrode. A high electric potential is applied to this electrode in pulses so that, at each pulse, a section of the ion beam is deflected and accelerated into the TOF analyser. After reflection by the reflectron, the ions are detected at a microchannel plate multipoint collector. The reflectron is used mainly to increase the time intervals at which successive m/z values are detected at the collector. The quadrupole is operated in the narrow bandpass mode for MS/MS and in its wide bandpass mode for obtaining a full spectrum by the TOF analyser.

HYBRID ORTHOGONAL-TOF INSTRUMENTS

Introduction

The *Back-to-Basics* sections on *Time-of-Flight Optics* and *Orthogonal Time-of-Flight* are relevant to the present section.

With Time-of-Flight (TOF) analysers, the time taken for ions to travel the length of an evacuated tube is used to deduce the m/z values of the ions and so obtain a spectrum. The ion beam may start from an ion source and be directed straight into the analyser, as shown in Figure 1a for a simple MALDI/TOF apparatus. This is the in-line or stand-alone TOF instrument and, as shown in the more extended description, it may be somewhat more complicated to enable MS/MS measurements to be made. Alternatively, an ion beam produced by some other analyser may be deflected into a TOF analyser placed at right angles (orthogonal) to the beam (see *Orthogonal TOF*), as shown in Figure 1b. This arrangement constitutes the basis of a hybrid TOF instrument. For either the in-line or hybrid analysers, the ions injected into the TOF section must all begin their flight down the TOF tube at the same instant if arrival times of ions at a detector are to be used to measure m/z values (see *Time-of-Flight Optics*). For the hybrid TOF instruments, the ion detector is usually a microchannel plate ion counter (see *Multipoint Collectors*).

In this section of *Back-to-Basics*, the analyser layouts for three hybrid instruments are described only briefly. More extensive treatments of Q-TOF, LCT (or LC-TOF with Z-spray), and AutoSpec-TOF (Sector-TOF) are provided in the relevant sections of *Back-to-Basics*.

(i) Q-TOF

The Q in Q-TOF stands for quadrupole (see *Quadrupole Ion Optics*). A Q-TOF instrument is normally used with an electrospray ion inlet and is used for measuring mass spectra directly so as to give molecular or quasimolecular mass information or it can be switched rapidly to MS/MS mode so as to examine structural features of ions. The analyser layout is discussed in Figure 2.

(ii) LCT

A liquid chromatograph (LC) is combined with a Time-of-Flight instrument (TOF) through a Z-SPRAY™ ion source. Two hexapoles are used to focus the ion beam before it is examined by a TOF analyser, as described in Figure 3.

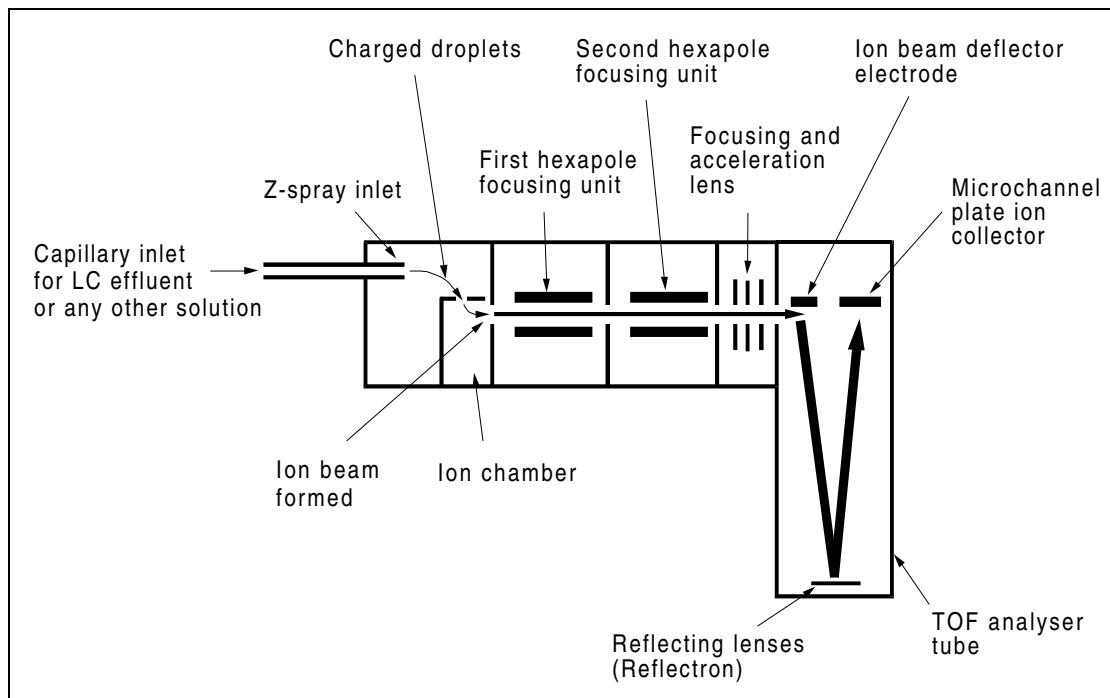


Figure 3 A solution containing the analyte of interest is sprayed from the end of a capillary by application of a high electric potential. The resulting charged droplets are stripped of solvent, and ions formed from analyte molecules travel into the mass spectrometer (Z-spray). The hexapoles do not separate ions according to m/z value but contain them into a beam. Finally, the slow moving ions are focused and accelerated through a potential of about 40 V before passing in front of a deflector plate (electrode). A large electric potential of several kV is applied to this electrode in pulses so that, at each pulse, a section of the ion beam is deflected and accelerated into the TOF analyser. After reflection by the reflectron, the ions are detected at a microchannel plate multipoint collector.

(iii) AutoSpec-TOF An AutoSpec-TOF mass spectrometer has a magnetic sector and an electron multiplier ion detector for carrying out one type of mass spectrometry plus a Time-of-Flight analyser with a microchannel plate multipoint ion collector for another type of mass spectrometry. Either analyser can be used separately or the two may be run in tandem (see Figure 4 for greater detail).

Conclusion

A Time-of-Flight analyser may be used alone or in conjunction with other analysers so as to give hybrid mass spectrometers. The hybrids provide advantages not attainable, or difficultly so, by the analysers used separately. When used in the hybrid mode, the TOF analyser is usually placed at right angles (orthogonal) to a main ion beam emanating from another analyser.

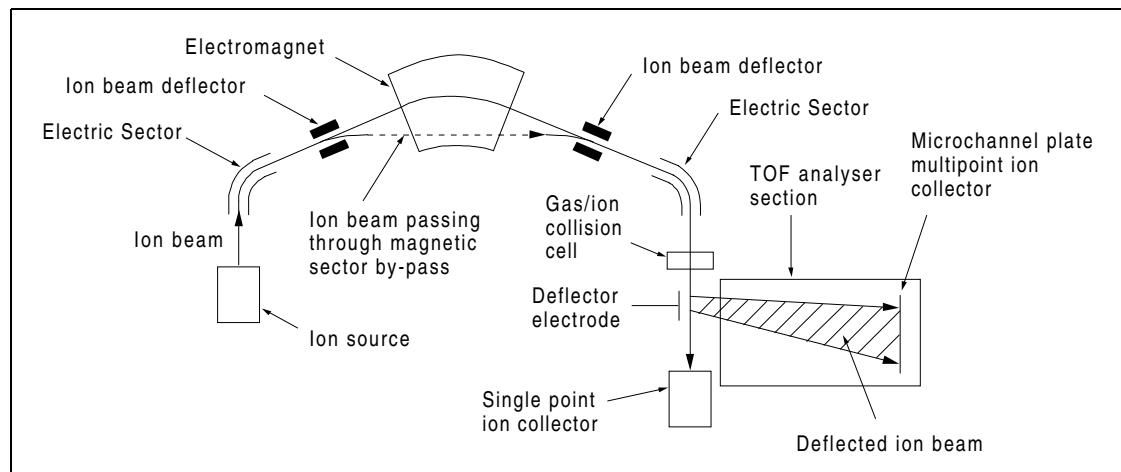


Figure 4 The main features of the AutoSpec-TOF hybrid layout. In one mode of operation, the instrument is used with the deflector electrode switched off and the main ion beam passing through the magnet sector for the separation of ions into their individual m/z values; a full mass spectrum is obtained by scanning the magnetic sector. In a second mode, the magnetic field is switched off and the beam deflectors transmit the whole ion beam through the magnet by-pass; the mass spectrum is obtained by application of a pulsed electric field to the deflector electrode, which allows m/z values of ions to be measured in the TOF analyser. In yet another mode, the ions of one m/z value can be selected from the main beam by the magnetic sector. A gas is placed in the collision cell and causes the ions to fragment. This fragmentation (MS/MS) spectrum is measured in the TOF analyser. Other modes of operation are possible.

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CHAPTER C2.1

HYBRID MAGNETIC SECTOR-TOF

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Quick Guide

- Magnetic/electric sector instruments are used to manipulate ion beams by making use of the deflection of charged species (ions) in magnetic or electric fields.
- For such instruments, the ions are accelerated from an ion source by using an electric potential of several thousand volts.
- By using the property that ions are deflected in a magnetic field in proportion to both the square root of their m/z values and the potential through which they have been accelerated, it is possible to measure the m/z values very accurately.
- The electric fields in such instruments are used to focus the fast moving ion beam according to the kinetic energies of the ions contained in it. This property allows ions of individual m/z values to be focused sharply before or after deflection in the magnetic field.
- This focusing action gives an ion beam, in which the m/z values can be measured so accurately that the resolution of a magnetic/electric sector instrument (separation of ions of different m/z values) is measured as a few parts per million, as against the more modest few parts per thousand in, say, a quadrupole or ion trap instrument.
- The ions are detected by an electron multiplier placed in line with the beam.
- Thus, ions are produced, deflected in a magnetic field, then focused in an electric field, and finally they are detected by an electron multiplier.
- An 'AutoSpec' instrument is just such a mass spectrometer.
- If, just before the ion beam reaches the electron multiplier, a 'pusher' electrode is used alongside it to deflect the beam at right angles (orthogonal) to its original direction into the flight tube of a Time-of-Flight sector (TOF analyser), the m/z values can be measured by the TOF section.
- The pusher electrode must be operated by placing very short pulses of electric potential on it. The short pulses are required to ensure that the ions are started off all at the same time along the TOF analyser, since the latter must time the flights of the ions very accurately in order to measure m/z values.

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- An AutoSpec (or any magnetic sector) /TOF hybrid has two means of measuring m/z values, one very accurately in a conventional magnetic/electric sector sense and the other, somewhat less accurately in a Time-of-Flight sense.
- This hybrid is used in one form to measure highly accurate m/z values so as to give excellent elemental compositions of ions and therefore molecular formulae from molecular ions and, in the other form, it is used to obtain MS/MS data at high resolution.
- Mixtures of substances can be examined without the need for initial chemical or analytical separation.
- The hybrid has other advantages of sensitivity, low signal-to-noise ratio, fast switching between MS and MS/MS modes, use with continuous or pulsed ion sources and use with high or low energy collision induced ion decomposition.

Summary

By having a magnetic/electric sector instrument in conjunction with an orthogonal Time-of-Flight instrument a hybrid mass spectrometer is produced. The m/z values of ions can be measured in MS mode at high resolution so as to give elemental compositions of ions or at somewhat lower resolution in MS/MS mode to give structural information about the ions. Mixtures of substances can be investigated without the need for initial separation of the individual components by, for example, liquid or gas chromatography.

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HYBRID MAGNETIC SECTOR-TOF

Introduction

The hybrid mass spectrometer formed by using a magnetic/electric sector instrument coupled with a Time-of-Flight instrument is termed here the AutoSpec-TOF, after the terminology of its manufacturer. However, the principles used could be any form of hybrid, in which a magnetic sector mass spectrometer precedes a Time-of-Flight mass (TOF) spectrometer. The principles lying behind the uses of magnetic/electric sectors and TOF instruments have been extensively described in these *Back-to-Basics Guides*. Accordingly, for much of the present guide, it is assumed that the other guides have been read (see: *Ion Optics of Magnetic/Electric Sector MS* and *Orthogonal Time-of-Flight Optics*).

In the magnetic sector/TOF hybrid, ions produced in an ion source pass through the magnetic sector first and then may or may not enter the TOF section, depending on how the hybrid is being operated. The hybrid can be used as two separate instruments or as two instruments in conjunction with each other.

In general terms, the main function of the magnetic/electric sector section of the hybrid is to be able to resolve m/z values differing by only a few parts per million. Such accuracy allows highly accurate measurement of m/z values and therefore affords excellent elemental compositions of ions; if these are molecular ions, the resulting compositions are in fact molecular formulae. This is the usual MS mode. Apart from accurate mass measurement, full mass spectra can be obtained also. The high resolution separation of ions also allows ions having only small mass differences to be carefully selected for MS/MS studies.

Also in general terms, the TOF part of the hybrid is used for mostly for MS/MS studies, in which ions produced in the magnetic sector are collided with neutral gas molecules to induce decomposition (see: *Collision Induced Metastable Ions* in these guides). In this mode the instrument produces much better resolved product ion spectra than can be attained in simple magnetic sector instruments.

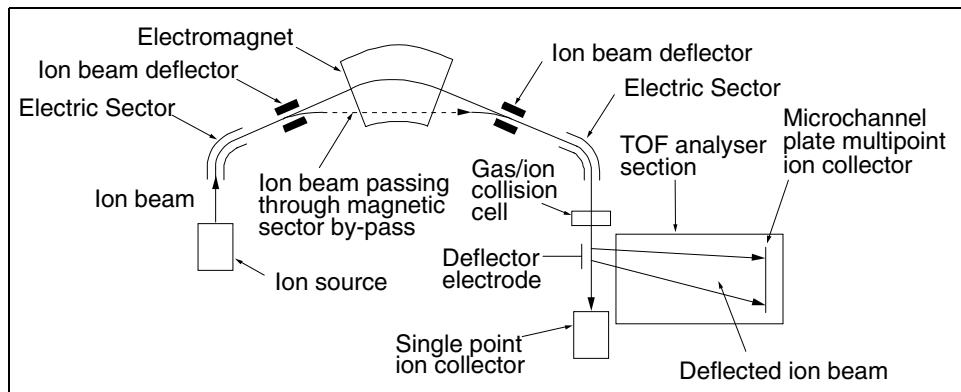


Figure I The main features of an AutoSpec-TOF hybrid layout. In one MS mode of operation, the instrument is used with the ion beam deflector electrodes switched off and the main ion beam passing through the magnet sector so as to separate the ions into their individual m/z values; a full mass spectrum is obtained by changing the magnetic field strength. In a second MS mode, the magnetic field is switched off and the beam deflectors transmit the whole ion beam through the magnet bypass; the mass spectrum is obtained by application of a pulsed electric field to the deflector electrode, which causes the ionic m/z values to be measured in the TOF analyser. In an MS/MS mode, the ions of one m/z value can be selected from the main beam by the magnetic sector. A gas is placed in the collision cell and causes the selected ions to fragment. This fragmentation spectrum is measured in the TOF analyser. Other modes of operation are possible.

A further important property of the two instruments concerns the nature of any ion sources used with them. Magnetic sector instruments work best with a continuous ion beam, as produced with, say an electron ionization or chemical ionization source. Sources that produce pulses of ions, such as with laser desorption or radioactive (Californium) sources, are not compatible with the need for a continuous beam. However, these 'pulsed' sources are ideal for the TOF analyser because, in such a system, ions of all m/z values must begin their flight to the ion detector at the same instant in order that their flight times can be measured accurately. Therefore, a magnetic sector/TOF hybrid can be used with a wide variety of ion sources.

In the following discussion, use of the magnetic sector or TOF sector separately is discussed briefly first and then the hybrid uses are exemplified.

The AutoSpec-TOF Ion Optics

The arrangement of magnetic and electric fields is shown in Figure 1.

The Magnetic/Electric Sector Section

For this instrument there are actually two electric sectors associated with the magnetic sector. If, for the moment, the TOF section is ignored, it can be seen that the remainder of figure 1 reveals a magnetic/electric sector instrument (the AutoSpec). Ions are accelerated from an ion source through an electric field of several thousand volts. This continuous ion beam is then deflected towards the magnetic sector by the first electric sector. No ion separation occurs in the electric sector stage but the beam is focused for kinetic energy. In the following magnetic analyser (sector), the ions are deflected according to their m/z values. By changing the magnetic field strength, ions of individual m/z values are selected and pass into the second electric sector, in which they are again focused and deflected. Again, no separation by m/z values occurs at this stage, all the separation into m/z values having occurred in the magnetic sector. The ions then pass through an 'empty' gas collision cell and into a single point ion collector (an electron multiplier). Thus, this section can be operated as a magnetic sector instrument having an electric sector (a high resolution mass spectrometer). The magnetic field can be changed continuously so that ions of successive m/z values are selected one after the other and pass into the ion detector. This mode gives a full mass spectrum.

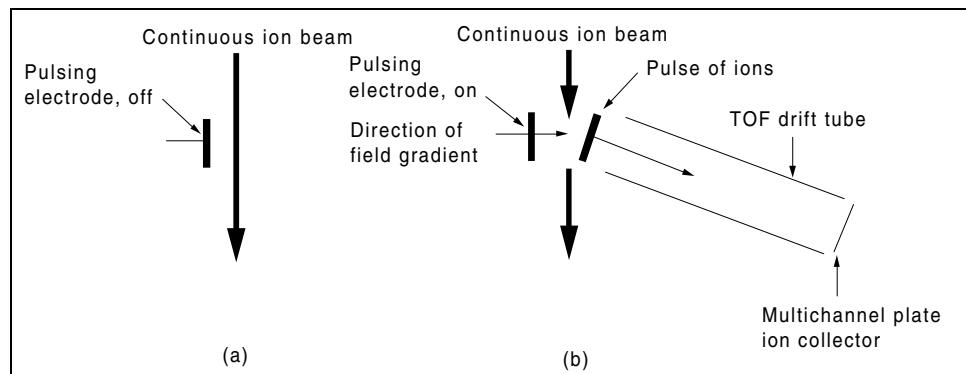


Figure 2 In (a), the pulsing electrode is switched off and a continuous ion beam passes by it. In (b), the electrode has been pulsed for a few nanoseconds, with a field gradient at right angles to the main beam. This has caused a short section of the ion beam to detach and to travel in the direction shown. The detached segment of the main beam enters the flight tube of a TOF instrument. The m/z values are determined from the times taken for the ions to reach the microchannel plate ion collector after initiation of the pulse.

By including a mass measurement standard substance, the mass spectrometer can be operated at high resolution to give accurate masses of the ions. These accurate masses give the elemental compositions of the ions. If they are molecular ions, the elemental composition is the molecular formula. The mass spectrometer is then being operated as a high resolution magnetic/electric sector instrument. It is in a high resolution MS mode.

Alternatively, ions of any one selected m/z value can be chosen by holding the magnetic field steady at the correct strength required to pass only the desired ions; any other ions are lost to the walls of the instrument. The selected ions pass through the gas cell and are detected in the single point ion collector. If there is a pressure of a neutral gas such as argon or helium in the gas cell then ion-molecule collisions occur with decomposition of some of the selected incident ions. This is the MS/MS mode. However, without the orthogonal TOF section, since there is no further separation by m/z value, the new ions produced in the gas cell would not be separated into individual m/z values before they reached the detector. Before the MS/MS mode can be used, the instrument must be operated in its hybrid state, as discussed below.

The Time-of-Flight Section

The TOF analyser may be used in its 'stand alone' mode just as the magnetic sector could be used. For this usage, the magnetic sector is unnecessary because all of the ions are required for the TOF analyser and not just selected ones. Accordingly, the AutoSpec uses two beam deflectors to bypass the magnetic sector (Figure 1). Now, ions produced in the source are accelerated again through several thousand volts and focused as before for kinetic energy in the first electric sector. The beam deflector passes the ion beam outside the magnetic field and into a second beam deflector, which redirects the beam into the second electric sector. Thus, the beam deflectors act like a bypass for the ion beam and no separation of the ions into individual m/z values can occur. All of the ions head towards the single point collector.

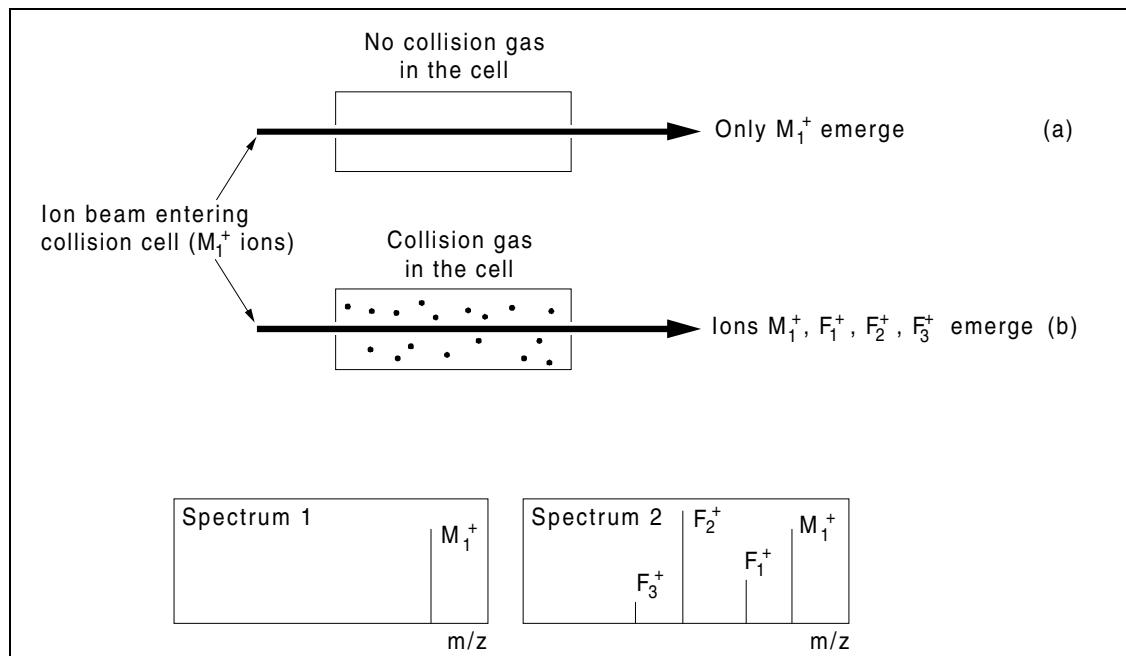


Figure 3 The diagram represents a beam of M_1^+ ions from the magnetic sector entering a gas collision cell. In (a), there is no collision gas in the cell so only M_1^+ ions emerge and the TOF analyser would give spectrum 1. In (b), there is collision gas in the cell. Now ion-molecule collisions lead to some fragment ions (F_1^+ , F_2^+ , F_3^+) being formed and these, along with undecomposed M_1^+ ions leave the collision cell to give spectrum 2, as measured by the TOF analyser. The hybrid has given an MS/MS result.

So far as the orthogonal TOF section is concerned, there is simply an ion beam passing the end of the analyser section (it should be remembered that the vacuum is continuous from the ion source right through the magnetic sector and into the TOF sector). The TOF analyser requires that, for measurement of m/z values, all ions must start their journey down the flight tube at the same instant. Therefore, there is a deflector or pusher electrode placed alongside the beam of ions passing the end of the TOF analyser (Figure 1). A very short electric field pulse of several thousand volts is applied to this electrode. This causes a small section of the beam to be deflected and accelerated into the TOF section (Figure 2). This small section of the beam contains a representative selection of the ions in the original ion beam but, now, the ions have all been started off down the TOF flight tube at the same instant (the pulsed electric field was like a runners' starter gun in a race). Ions travel down the flight tube at different speeds (depending on the square roots of their m/z values) and reach a microchannel multipoint ion collector at different times. The flight times give the m/z values. By applying a pulse to the deflector electrode, a full mass spectrum of the ions in the beam can be obtained. This constitutes operation of the TOF section in an MS only mode. The resolution in the TOF mode is quite high but is not as good as for the magnetic sector MS mode.

Because the resolution in the MS mode is better with the magnetic sector, this is the preferred mode of operation for obtaining a routine mass spectrum or when accurate mass measurements need to be made. However, this is not always the case. The ion beam emerging from the ion source may be unsteady, depending on the source, or it may be pulsed as with laser desorption ionization. Obtaining a full mass spectrum from the magnetic sector in these conditions is at best difficult and at worst virtually impossible. The TOF section has an operational speed of just a few microseconds for a complete mass spectrum. Thus, for a fluctuating or pulsed ion source, the TOF analyser can capture a full mass spectrum in as short a time as the fluctuations or pulses. Further, the TOF sector can be operated at a speed of several kHz and it is possible to accumulate several thousand spectra in one second. This is an ideal arrangement for smoothing out fluctuations in ion yield or for capturing mass spectra from pulsed ion sources and the TOF section alone may be used for MS mode operation in some circumstances.

Like the magnetic sector, the TOF section by itself is not capable of MS/MS operation but, allied with the sector, the two make an excellent MS/MS instrument.

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Operation of the Combined Magnetic and TOF Sectors as a Hybrid Instrument

This mode of operation is used for MS/MS studies. For mass spectrometry, molecular ions are valuable because their m/z values give molecular mass information and, if measured accurately, they give molecular formulae. However, fragmentation of the molecular ions is useful because it gives information about the structure of the sample put into the mass spectrometer. Electron ionization gives both molecular and fragment ions so that it provides two lots of information. Contrariwise, many ionization sources, such as those based on chemical, electrospray, fast atom bombardment or laser ionization, give mostly or entirely molecular or quasimolecular ions and there is no fragmentation. This means that structural information is lost. To offset this drawback to the so-called 'soft' ionization methods, the molecular ions are made to decompose (fragment) by passing them through a cell, which operates at a gas pressure somewhat above that for the remainder of the mass spectrometer. Collisions between ions and the gas molecules (often helium or argon) causes some of the molecular ions to form fragment ions, viz., a mass spectrum is produced and provides structural information. This is the basis of MS/MS, in which two mass spectrometers are used, as in the AutoSpec-TOF hybrid.

In operation, the magnetic section of the hybrid is used to select ions of a particular m/z value. For example, if a mixture of two substances gives two molecular ions, M_1 and M_2 , the magnetic sector is used to select one or the other. The selected ions collide with gas in the collision cell (Figure 1) and some of them decompose to yield fragment ions, say F_1 , F_2 and F_3 . Thus, a stream of ions M_1 (some of which have not been decomposed) plus F_1 , F_2 and F_3 leaves the collision cell (Figure 3). If this beam went straight to the single point ion collector, there would be no separation into the individual m/z values and it would not be possible to measure their m/z values. However, by pulsing the deflector electrode placed just after the collision cell, a section of the beam is sent off orthogonally down the TOF analyser tube, which does separate them according to m/z value from the length of time they take to reach the multipoint microchannel plate collector (Figure 1). Therefore, molecular ions and fragment ions are obtained in this MS/MS mode.

A big advantage of the AutoSpec-TOF hybrid lies in its ability to examine ions at high resolution. Supposing in the above example, the two molecular ions M_1 and M_2 had the same integer m/z values. At low resolution, the two ions could not be distinguished. However, their accurate masses might differ by, say, 0.0005 mass units. Now, at high resolution, the two sets of ions can be separated in the magnetic sector and it again becomes possible to distinguish M_1 and M_2 .

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Other Advantages of the Hybrid

The hybrid can be used with EI, CI, FI, FD, LSIMS, APCI, ES and MALDI ionization/inlet systems. The nature of the hybrid leads to high sensitivity in both MS and MS/MS modes and there is rapid switching between the two. The combination is particularly useful for biochemical and environmental analyses because of its high sensitivity and the ease of obtaining MS/MS structural information from very small amounts of material. The structural information can be controlled by operating the gas cell at high or low collision energies.

Conclusion

The AutoSpec-TOF hybrid mass spectrometer combines the advantages of a magnetic/electric sector instrument with those of Time-of-Flight, to give a versatile instrument capable of MS or MS/MS at high or low resolution.

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CHAPTER C2.2

HYBRID HEXAPOLE-TOF

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Quick Guide

- Basically, this system is very similar to that of a Q-TOF instrument but without the initial quadrupole section.
- An ion beam may be produced from a number of different sources but, generally for this instrument (used for biochemical examination of thermally unstable, large molecules), an atmospheric pressure inlet such as APCI or ES would be used. These may be operated with liquid inlets from chromatographic columns or simply from static solutions.
- The atmospheric pressure ionization inlets produce a stream of cations or anions, which have been formed at about room temperature and have little excess of thermal energy. Consequently, these ions are very stable and exhibit little or no fragmentation (see Back-to-Basics guides on combined inlet/ionization systems).
- In the Hybrid Hexapole-TOF instrument, these ions are collimated through two consecutive hexapole sections, each operating at different pressures.
- Unlike a quadrupole, a hexapole assembly is incapable of separating ions according to their m/z values. However, it is capable of accepting an ion beam and ensuring that the beam is kept as narrow as possible and remains on a straight line track.
- The gas pressure in the first hexapole section is influenced by gas leaking in from the inlet system and is higher at about 10^{-4} mbar than is desirable if excessive ion/neutral collisions are to be prevented. Therefore, the first hexapole is separated from the second hexapole section by a small orifice, which allows ions to pass through.
- Differential pumping of the two hexapole sections keeps the second at a pressure of about 10^{-5} mbar. The two consecutive hexapole sections are sometimes described as 'bridges' between the pressure in the inlet and the following vacuum in the TOF mass analyser.
- After passing through the hexapoles, the ion beam emerges in front of a 'pusher' electrode built into the end of the TOF analyser.
- The TOF analyser is positioned at right angles (orthogonal) to the incoming ion beam.

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- Application of a pulse of high electric potential (about 1 kV) to the pusher electrode over a period of about 3 μ s causes a short section of the ion beam to be detached and accelerated into a Time-of-Flight (TOF) analyser. A positive potential is used to accelerate positively charged ions and vice versa for negative ions.
- The detached section of ions sets off along the TOF analyser with the ions having velocities proportional to the square roots of their m/z values.
- A reflectron is a special device designed to reverse the direction of travel of ions as they near the end of the Time-of-Flight tube by having an opposing electric field gradient. At some point in the reflectron, the ions are stopped and then accelerated back out. The ions return through the flight tube but along a slightly different trajectory to their initial one.
- The total trajectory of the ions is approximately V-shaped, the top of one leg of the V being the position of the pusher electrode and the top of the other being the position of the ion collector (a microchannel plate detector).
- The reflectron increases the spatial separation of the ions of different m/z values by making them travel up and down the flight tube so that their trajectory is twice that which it would be if the ions simply passed once along the tube from one end to the other. The reflectron also narrows the energy spread for individual m/z values.
- The TOF analyser provides the full mass spectrum of all the ions in the main ion beam at the time the pulse of electric potential was applied, m/z values being derived from the flight times of the ions along and back along the TOF analyser.
- The mass range is usually up to about 10,000 mass units (Daltons).

Summary

The system is a modified Q-TOF and is ideal for examining mass spectra of substrates dissolved in solvents as occurs with substances, which emerge from a liquid chromatographic column and pass into an atmospheric pressure ionization source. By operating two hexapole sections as a bridge between the atmospheric pressure inlet/ion source and an orthogonal TOF analyser, a full mass spectrum may be obtained of all ions from the ion source and therefore of substrates eluting from an LC column. The solution used may come from a static supply and not just from an LC column.

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HYBRID HEXAPOLE-TOF

Introduction

The term Hybrid-Hexapole-TOF is used to describe a type of hybrid mass spectrometer system, in which a hexapole assembly in conjunction with a Time-of-Flight analyser (TOF) is used to obtain mass spectra of ions emerging from an atmospheric pressure inlet/ion source (i.e., the Micromass LCT). The latter source is frequently used for dealing with solutions eluting from a liquid chromatographic column. Thus, as a substrate emerges from an LC column, it frequently passes through some sort of detector (UV/visible, refractive index, etc.) to reveal when substrates are emerging. Although these detectors reveal when a substrate is eluting, they are not generally useful for giving the sort of detailed structural information available from a mass spectrum. To obtain such a spectrum, first the solvent must be stripped from the substrate, which itself needs to be ionized. These two operations of desolvation and ionization are conveniently effected concurrently by using an atmospheric pressure inlet/ionization source, such as APCI or ES (see Back-to-Basics guides on *Atmospheric Pressure Chemical Ionization* and *Electrospray Ionization*). Once the solvent has been stripped away and the substrate (solute) has been ionized, the ions are examined for m/z values by a Time-of-Flight (TOF) analyser placed orthogonally to the main ion beam (see: *Time-of-Flight Analysers* in these guides). The solution to be examined does not need to be from an LC column but equally well could be simply a static solution in a container. Other aspects of this hybrid appear in these guides under the heading *Hybrid TOF instruments*.

Inlet Systems

The LCT (LC-TOF) instrument was designed specifically for use with the effluent flowing from liquid chromatographic columns but can be used also with static solutions. The initial problem with either of these inlets revolves around how to remove the solvent without affecting the substrate (solute) dissolved in it. Without this step, on ionization, the large excess of ionized solvent molecules would make it difficult if not impossible to observe ions due only to the substrate. Combined inlet/ionization systems are ideal for this purpose. For example, dynamic fast atom bombardment, plasmaspray, thermospray, Atmospheric Pressure Chemical Ionization (APCI) and electrospray (ES) have all been used (see: relevant Back-to-Basics guides) but only the last two are now common. The ES inlet has evolved into the highly efficient Z-spray (see: *Z-spray combined inlet/ionization system*). After passing through such inlets, most of the solvent has been removed.

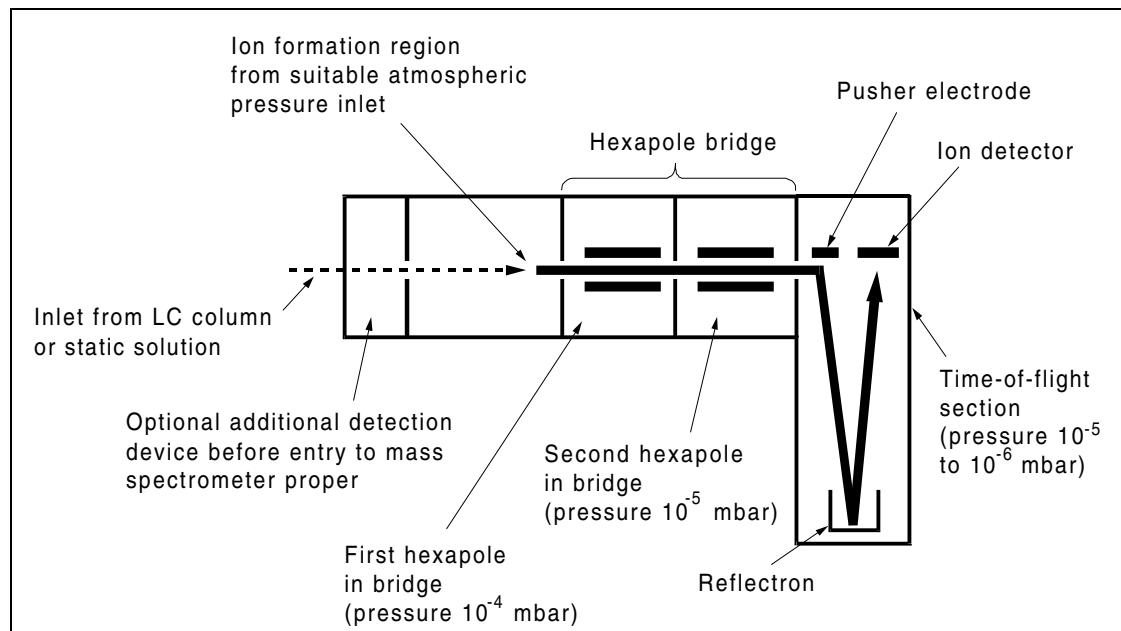


Figure I A diagrammatic representation of a Hexapole-TOF instrument, showing the consecutive arrangement of solution inlet at atmospheric pressure, the ion source (in which the pressure begins to be reduced), the hexapole bridge (in which the pressure is further reduced) and finally the m/z measurement in the TOF section (in which the vacuum is the highest). The path of the ion beam is shown as a bold black line. After reaching the pusher electrode, sections of the beam are accelerated into the TOF unit, where m/z separation takes place. After passing through the reflectron, the ions are detected at a microchannel plate collector.

Residual solvent and ions from the substrate are passed into the beginning of the Hexapole-TOF instrument proper. At this point, background air pressure and the remaining few solvent molecules must be removed, whilst keeping the substrate ion beam intact and on line to pass into the Time-of-Flight mass analyser. This connection between the inlet and the analyser is composed of a hexapole ‘bridge’ (Figure 1).

In this mode of operation with atmospheric pressure inlet systems, other measuring devices can still be used at the end of an LC column. Thus, the common UV/visible, refractive index, radioactive and light scattering devices can be used without affecting the performance of the mass spectrometer, thereby giving more analytical information (Figure 1).

The Hexapole Bridge

A quadrupole mass analyser may be used in two modes, called narrow and wide band pass. In the first, two opposed poles have an applied radio frequency voltage applied, with the other two opposed poles having a constant DC voltage. Depending on the voltages and the frequency of the RF field, ions of selected m/z values pass right through the four poles but others cannot. The device is used as a mass analyser. In the wide band pass or RF only mode, all four poles have only an applied radio frequency electric field and all ions are transmitted so that the quadrupolar assembly acts as an ion guide, containing and directing an ion beam along the length of the poles. A hexapole assembly of rods (poles) is built similarly to the quadrupole but now there are six rods evenly spaced around a central axis (Figure 2). The hexapole cannot act as a mass filter by applying a DC field and is used only in its all RF mode, in which it allows all ions in a beam to pass through whatever their m/z values. In doing this, the ion beam is constrained so that it leaves the hexapole as a narrow beam. This is important because the ion beam from the inlet system tends to spread due to mutual ion repulsion and collision with residual air and solvent molecules. By injecting this divergent beam into a hexapole unit, it can be ‘refocused’. At the same time, vacuum pumps reduce the background pressure to about 10^{-4} mbar (Figure 1). The pressure needed in the TOF analyser is about 10^{-5} to 10^{-6} mbar and, usually, a second hexapole unit is provided to maintain the ion beam on track and collimated. The passage from the first hexapole into the second is through a very small diameter orifice, which allows the passage of the ion beam but restricts entry of gas molecules. Thus, a second vacuum pump deals with residual gas in the second hexapole chamber, bringing down the pressure to about 10^{-5} mbar.

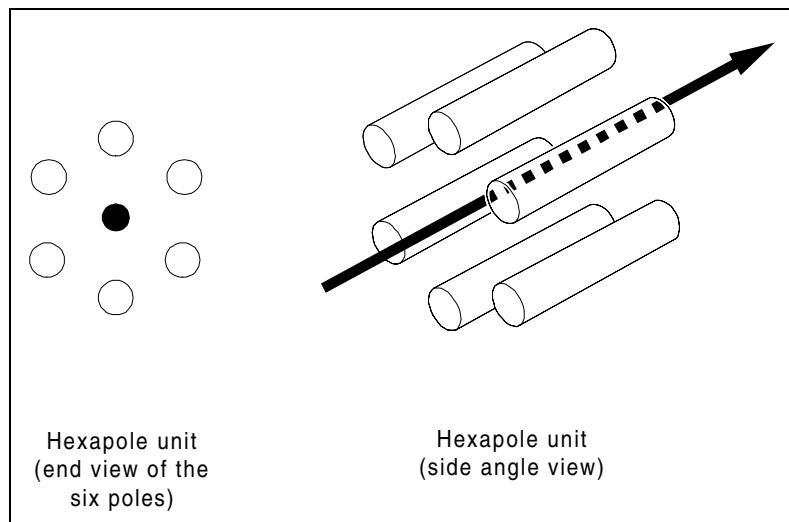


Figure 2 A diagrammatic representation of a hexapole unit. The left-hand of the figure shows the end view of the unit, the ends of the six poles being represented as open circles and the ion beam by the black circle. The right-hand of the figure shows an angled side view of the unit, showing the six parallel poles (or rods), with the ion beam passing through the middle of them. If it is the first hexapole then the emergent ion beam passes through a narrow orifice into the second hexapole unit. If it is the second hexapole unit then the ion beam passes through a narrow orifice into an acceleration (electric) lens before passing in front of the pusher electrode of the TOF tube. Note that the small lens system is not shown in Figure 1 for the sake of clarity.

After this second hexapole stage, the ion beam is focused and accelerated slightly and passed into the TOF analyser (Figure 1). It can be seen that the two hexapoles act as a means of channelling the ions from the inlet near atmospheric pressure to the TOF analyser under high vacuum but without introducing any selection by m/z value. It is for this reason that the hexapoles are said to bridge the inlet and analyser sections of the mass spectrometer.

Time-of-Flight Analyser

If ions of different m/z values are ‘instantaneously’ accelerated by an electric field, they attain velocities determined by their m/z values and the accelerating field to which they have been subjected. After ‘flying’ along an evacuated tube, the ions arrive at a detector in proportion to the velocities they have, the faster ions arriving before slower ones. Because an ion velocity depends upon its m/z value, the ions of smaller m/z value arrive first and those of larger value arrive last. By measuring the ‘flight time’ of the ions along a TOF tube (analyser), the m/z values can be deduced and therefore a mass spectrum can be measured. Among the advantages of TOF measurement of mass spectra is the very short interval of time needed to measure a full mass spectrum. Typically, a few microseconds is all that is needed to scan from say 1 to 2,000 mass units. On the human timescale, this speed of scanning appears to be instantaneous. For sake of illustration, a TOF analyser could be likened to a camera taking snapshots of the m/z values of an assembly (beam) of ions; the faster the repetition rate at which the camera shutter is clicked the more mass spectra can be taken in a very short time. For TOF analysers, it is not uncommon to measure several thousand mass spectra in one second! All such spectra can be added to each other digitally, a process that leads to improvements in signal-to-noise ratio in the final accumulated total.

Clearly, the longer the flight tube, the longer it will take for ions to traverse it. For two ions of velocities v_1 and v_2 , their flight times will be t_1 and t_2 for a flight path of length d . If the flight path is doubled in length, the flight times become $2t_1$ and $2t_2$ and the difference in arrival times for the two ions at a detector is doubled, viz, it changes from (t_1-t_2) to $2 \times (t_1-t_2)$. As differences in flight arrival times are typically measured in nanoseconds for a flight tube of 50 cm length, then doubling this length doubles the differences and makes their measurement that much easier. In effect, the resolution of the instrument is greatly improved by making the ions pass twice along a TOF flight tube instead of them passing only once along it before reaching the detector (see Figure 1). The device that reflects the ions is called a reflectron.

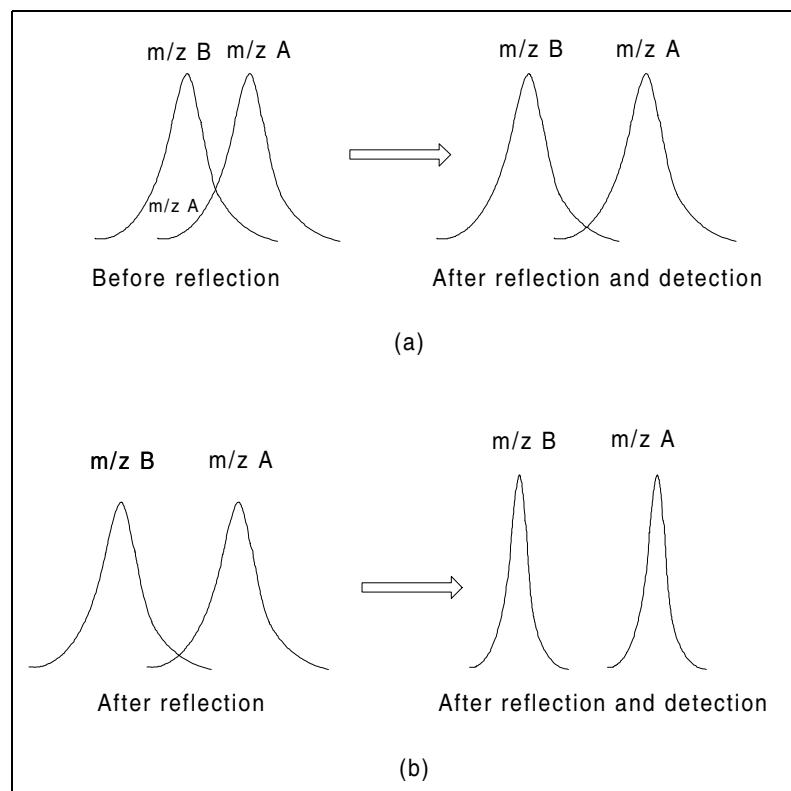


Figure 3 In (a), two peaks are shown, representing kinetic energy distributions for two sets of ions of m/z values A and B. The two peaks are shown as overlapping extensively. If there were no improvement, the resolution of the instrument would be quite poor. After doubling the flight path by use of the reflectron, the separation of the tops of the peaks is doubled also and this improves their resolution. In (b), a second effect of the reflectron is shown. Now, the kinetic energy spread appears to be much narrower and the resolution of the peaks at m/z A, B is improved again. The combination of doubling the length of the flight path and decreasing the separation of ions of each m/z value leads to greatly improved instrumental resolution of m/z values.

There is another advantage of the reflectron. Ions of one particular m/z value should all have the same velocity when travelling along the flight tube. However, because of various small inhomogeneities in the electric optics and other applied fields, there will be a small spread of velocities. Figure 3a, b shows typical spreads in velocities (and therefore in kinetic energies) for two sets of ions centred at m/z A and m/z B. Without the reflectron, these ions would reach the detector over small intervals of time rather than tightly bunched at two specific times. If the time difference between arrivals of the ions at m/z A and the following ones at m/z B is only small, then there will be some overlap between the slowest ions of the first m/z value and the fastest ions of the second m/z value (see Figure 3a). In effect, this means that the ions of different m/z value are inadequately separated - the resolution of the analyser is impaired. In the reflectron, faster ions travel further into it compared with any slower ones before being reversed and sent back out. Thus, the slower ions get a chance to 'catch up' with the faster ones so that, when both slower ones and faster ones reach the detector, they do so at about the same time. This effect appears to reduce the velocity spread in the ions of any one particular m/z value and leads to a major improvement in the resolution of the analyser (Figure 3b).

Operation of the Hybrid

The effluent from an LC column or from a static solution supply is passed into an atmospheric pressure inlet/ion source (Figure 1), where ions are produced. The ions are transmitted rapidly to the TOF analyser, which is set to record a mass spectrum at a rate of say one every 30-40 microseconds. Since most of the background of air and solvent molecules has been removed, when there is no solute present, there is only a very small amount of background 'noise'. When a solute appears, mass spectra are obtained.

Some Advantages of the Hybrid

Ions formed in an electrospray or similar ion source are said to be 'thermalized', which is to say that their distribution of internal energies is close to that expected for their normal room temperature ground state. Such ions have little or no excess of internal energy and exhibit no tendency to fragment. This is an enormous advantage for obtaining molecular mass information from the stable molecular ions although there is a lack of structural information.

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By being able to obtain an unequivocal relative molecular mass, or even a molecular formula derived from that mass, the hybrid mass spectrometer becomes a powerful tool for investigating single substances or mixtures of substances. With an APCI inlet, fragmentation can be induced so as to give structural information (see the relevant Back-to-Basics guides).

Other instrumental advantages include its high sensitivity and a linear mass scale to m/z 10,000 at full sensitivity. The linearity of the mass scale means that it is only necessary to calibrate the spectrometer using single or sometimes two known mass standards. Some calibration is necessary because the start of the mass scale is subject to some instrumental ‘zero offset’. The digitized accumulation of spectra provides a better signal-to-noise ratio than can be obtained from one spectrum alone.

Summary

In one instrument, ions produced from an atmospheric pressure ion source can be measured. If these are molecular ions, their relative molecular mass is obtained and often their elemental compositions. Fragment ions may be produced by suitable operation of an APCI inlet, so as to obtain a full mass spectrum for each eluting substrate. The system can be used with the effluent from an LC column or with a solution from a static solution supply. When used with an LC column, any detectors generally used with the LC instrument itself can be still be included, thereby giving the hybrid the considerable advantage of molecular mass information and a mass spectrum as well as, say UV/visible spectra from a diode array detector sited in front of the mass spectrometer inlet system.

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Back to Basics Section C: Instruments

CHAPTER C2.3

HYBRID QUADRUPOLE-TOF

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Quick Guide

- A quadrupole analyser may be operated in either RF mode only or in RF/DC mode.
- In its RF only configuration (wide bandpass mode), all ions produced in an ion source and accelerated gently into the quadrupole analyser will pass through it, whatever their m/z values. The other mode is discussed further down the page.
- On emerging from the quadrupole, the ions are accelerated through about 40 V and focused. A ‘pusher’ electrode is sited alongside this focused ion beam.
- Application of a pulse of high electric potential (about 1 kV) to the pusher electrode over a period of about 3 μ s causes a short section of the ion beam to be detached and accelerated into a Time-of-Flight (TOF) analyser. A positive potential is used to accelerate positively charged ions and vice versa.
- A TOF analyser is placed at right angles (orthogonal) to the main ion beam and therefore the pusher electrode accelerates a short section of this beam into the flight tube of the TOF analyser.
- The detached section of ions sets off along the TOF analyser with the ions having velocities proportional to the square roots of their m/z values.
- Often, the TOF flight tube includes a reflectron.
- The reflectron is a special device designed to reverse the direction of travel of ions as they near the end of the flight tube by an opposing electric field gradient. At some point in the reflectron, the ions are stopped and then accelerated back out. The ions may return through the flight tube or along a slightly different trajectory to their initial one.
- Where the return path is different, the trajectory of the ions is approximately V-shaped, the top of one leg of the V being the position of the pusher electrode and the top of the other being the position of the ion collector (a microchannel plate detector).
- The reflectron increases the spatial separation of the ions of different m/z values by making them travel up and down the flight tube so that their trajectory is twice that which it would be if the ions simply passed along the tube from one end to the other. The reflectron also narrows the energy spread for individual m/z values.

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- The TOF analyser provides the full mass spectrum of all the ions in the main ion beam at the time the pulse of electric potential was applied, m/z values being derived from the flight times of the ions along their trajectory in the TOF analyser.
- If the initial quadrupole analyser is operated in its RF/DC mode (narrow bandpass mode), any one m/z value can be selected to pass right through the quadrupole analyser. Ions of all other m/z values are shut out.
- The main ion beam emerging from the quadrupole now has only ions of one selected m/z value. If the TOF is operating, the mass spectrum would consist of only one main peak (with peaks for isotopes).
- However, if before reaching the pusher electrode, the selected ions can be collided with a neutral gas in a hexapole device. The collision leads to some of the selected ions fragmenting so that the ions reaching the pusher electrode consist of some ions of the original selected m/z value plus ions of m/z values resulting from fragmentation. These ions travel along the TOF analyser and give an MS/MS product ion spectrum.

Summary

By operating a quadrupole analyser as a ‘gate’ together with an orthogonal TOF analyser, a full mass spectrum may be obtained of all ions from an ion source if the ‘gate’ is open. Alternatively, by selectively opening and closing the ‘gate’, ions of selected m/z values can be chosen for MS/MS studies. In either case, the TOF analyser is used to obtain the mass spectrum.

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HYBRID QUADRUPOLE-TOF

Introduction

The term Q-TOF is used to describe a type of hybrid mass spectrometer system, in which a quadrupole analyser (Q) is used in conjunction with a Time-of-Flight analyser (TOF). By using two analysers together (hybridized), distinct advantages accrue, which outweigh those of either analyser individually. In the Q-TOF, the quadrupole is used in one of two modes to select the ions to be examined; the TOF analyser measures the actual mass spectrum. A brief description of this hybrid appears in these guides under the heading Hybrid TOF instruments. For further information on the quadrupole or Time-of-Flight instruments themselves, please consult the guides, Quadrupole Optics and Time-of-Flight Optics. Hexapole assemblies are also used to help collimate the ion beams.

The Separate Quadrupole and Time-of-Flight Analysers

(i) A Quadrupole Assembly in Narrow Bandpass Mode

A quadrupole analyser may be operated in two main configurations of electric fields. If an RF potential is applied to two opposed poles (rods) and a DC potential is applied to the other two opposed poles then, by adjustment of the voltages and frequencies of the applied fields, ions of any selected m/z value injected at a small velocity into the front end of the quadrupole can pass right through the inter pole space and pass out at the other end. Ions of m/z values not selected, do not pass through but strike the poles (rods) and are lost. By continuously adjusting the voltages on the poles, ions of successive m/z values can be allowed to pass through the assembly in turn so as to give a mass spectrum of all of the ions injected into the front end (e.g., from an ion source). This configuration of electric fields is often termed the narrow bandpass mode and effectively acts as an electronic gate (sometimes erroneously called a mass filter). By opening or closing the gate, ions of selected m/z values can be allowed to traverse from one end of the quadrupole assembly to the other.

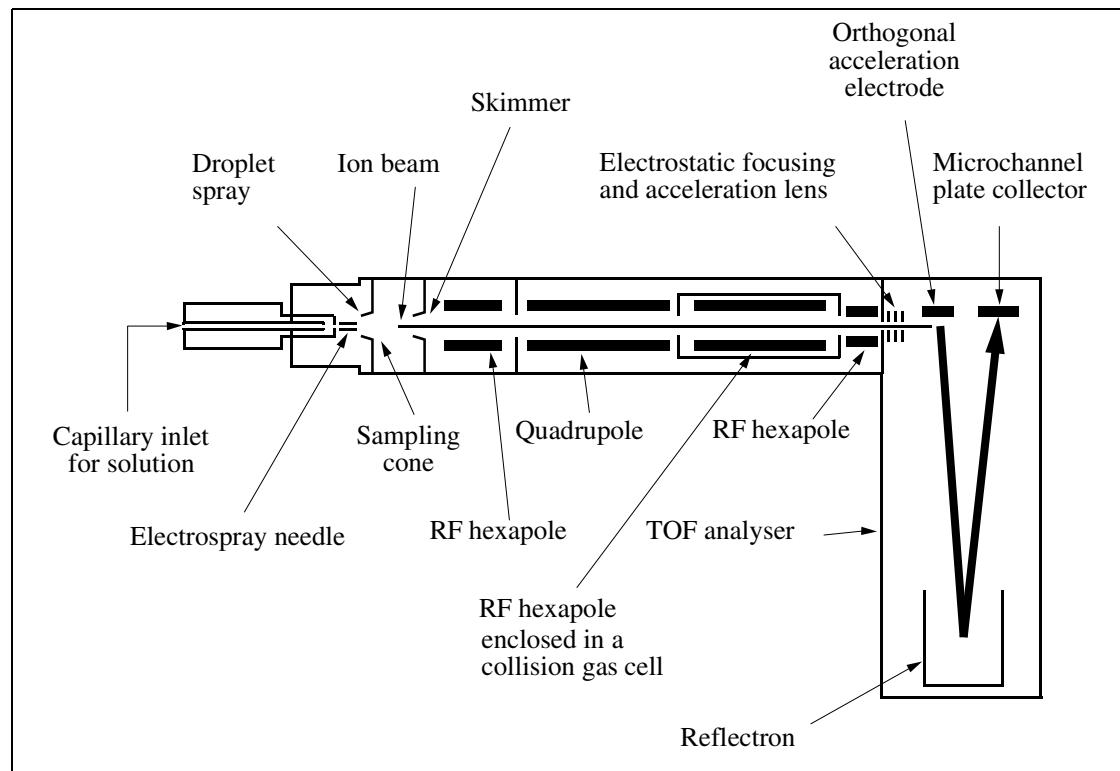


Figure I A schematic diagram of an orthogonal Q-TOF instrument. In this example, an ion beam is produced by electrospray ionization. The solution may be an effluent from a liquid chromatography column or may be simply a solution of an analyte. The sampling cone and the skimmer help to separate analyte ions from solvent. The RF hexapoles cannot separate ions according to m/z values and are used to help confine the ions into a narrow beam. The quadrupole can be made to operate in two modes. In one (wide bandpass), all of the ion beam passes through. In the other (narrow bandpass), only ions selected according to m/z value are allowed through and, for this configuration, the gas pressure in the middle hexapole is increased so that ions selected in the quadrupole are caused to fragment following collisions with gas molecules. In both modes, the TOF analyser is used to produce the final mass spectrum.

(ii) A Quadrupole in Wide Bandpass Mode

Alternatively, the quadrupole may be operated with only RF potentials applied to all four poles (rods). In this wide bandpass mode, no selection of ions occurs, the electric gate being effectively held open. Now, all ions produced in an ion source and injected into the front end of the quadrupole assembly traverse it and emerge from the other end. No ions are lost by striking the poles. The rod assembly acts to contain the ion beam so that it does not spread (the beam is collimated). Thus, all ions emerging from the quadrupole represent those being produced in the ion source. The wide and narrow bandpass modes may be switched very quickly so that, at one instant the quadrupole may be letting through selected ions of m/z value (say) 100 or m/z 200 or m/z 300 and so on. After rapidly switching to the wide bandpass mode, all of the ions of m/z 100, 200 and 300 are allowed through the quadrupole together.

Hexapoles

A hexapole assembly of rods (poles) is built similarly to the quadrupole but now there are three sets of opposed rods evenly spaced around a central axis. The hexapole cannot act as a mass filter by applying a DC field and is used only in its all RF mode. It is therefore a wide bandpass filter and is used to collimate an ion beam (like-charged particles repel each other and an electrically charged beam will tend to spread apart through mutual repulsion of ions unless steps are taken to prevent this).

Time-of-Flight Analyser

If ions of different m/z values are ‘instantaneously’ accelerated by an electric field, they attain velocities determined by their m/z values and the accelerating field to which they have been subjected. After ‘flying’ along an evacuated tube, the ions arrive at a detector in proportion to the velocities they have, the faster ions arriving before slower ones. Because an ion velocity (v) depends upon the inverse of the square root of its m/z value ($v^2 = 2zeV/m$), the ions of smaller m/z value arrive first and those of larger value arrive last. Thus, by measuring the ‘flight time’ of the ions along a TOF tube (analyser), the m/z values can be deduced and therefore a mass spectrum can be measured. Among the advantages of TOF measurement of mass spectra is the very short interval of time needed to measure a full mass spectrum. Typically, a few microseconds is all that is needed to scan from say 1 to 2000 mass units. On the human timescale, this speed of scanning appears to be instantaneous.

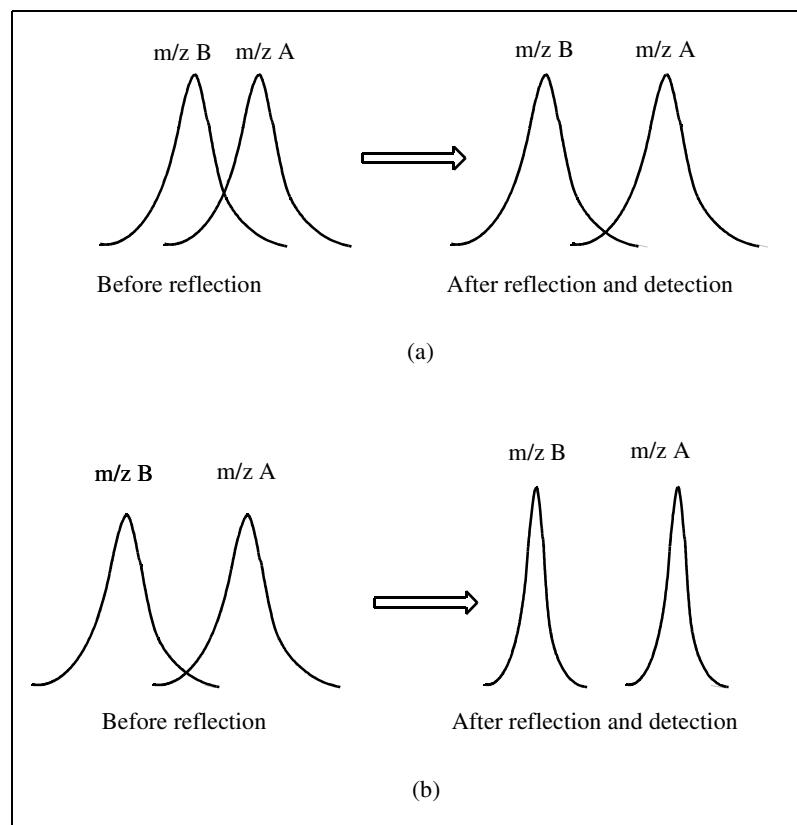


Figure 2 In (a), two peaks are shown, representing kinetic energy distributions for two sets of ions of m/z values A and B. The two peaks are shown as overlapping extensively. If there were no improvement, the resolution of the instrument would be quite poor. After doubling the flight path by use of the reflectron, the separation of the tops of the peaks is doubled also and this improves their resolution.

In (b), a second effect of the reflectron is shown. Now, the kinetic energy or velocity spread is much narrower and the resolution of the peaks at m/z A, B is improved again. The combination of doubling the length of the flight path and decreasing the velocity spread of ions of each m/z value leads to greatly improved instrumental resolution of m/z values.

For the sake of illustration, a TOF analyser could be likened to a camera taking snapshots of the m/z values of an assembly (beam) of ions; the faster the repetition rate at which the camera shutter is clicked the more mass spectra can be taken in a very short time. For TOF analysers, it is not uncommon to measure several thousand mass spectra in one second! All such spectra can be added to each other digitally, a process that leads to improvements in signal-to-noise ratio in the final accumulated total.

Clearly, the longer the flight tube, the longer it will take for ions to traverse it. For two ions of velocities v_1 and v_2 , their flight times will be t_1 and t_2 for a flight path of length d . If the flight path is doubled in length, the flight times become $2t_1$ and $2t_2$ and the difference in arrival times for the two ions at a detector changes from (t_1-t_2) to $2 \times (t_1-t_2)$, viz., it is doubled. As differences in flight arrival times are typically measured in nanoseconds for a flight tube of 50 cm length, then doubling this length doubles the differences and makes their measurement that much easier. In effect, the resolution of the instrument is greatly improved by making the ions pass twice along a TOF flight tube instead of them passing only once along it before reaching the detector (see Figure 2a). The device that reflects the ions is called a reflectron (see Figure 1).

There is another advantage of the reflectron. Ions of one particular m/z value should all have the same velocity when travelling along the flight tube. However, because of various small inhomogeneities in the electric optics and other applied fields, there will be a small spread of velocities. Figure 2a, b shows typical spreads in velocities (and therefore in kinetic energies) for two sets of ions centred at m/z A and m/z B. Without the reflectron, these ions would reach the detector over small intervals of time rather than tightly bunched at two specific times. If the time difference between arrivals of the ions at m/z A and the following ones at m/z B is only small, then there will be some overlap between the slowest ions of the first m/z value and the fastest ions of the second m/z value (see Figure 2a). In effect, this means that the ions of different m/z value are inadequately separated - the resolution of the analyser is impaired. In the reflectron, faster ions travel further into it compared with any slower ones before being reversed and sent back out. Thus, the slower ions get a chance to 'catch up' with the faster ones so that, when both slower ones and faster ones reach the detector, they do so at about the same time. The result is an apparent reduction in the velocity spread in the ions of any one particular m/z value and leads to a major improvement in the resolution of the analyser (Figure 2b).

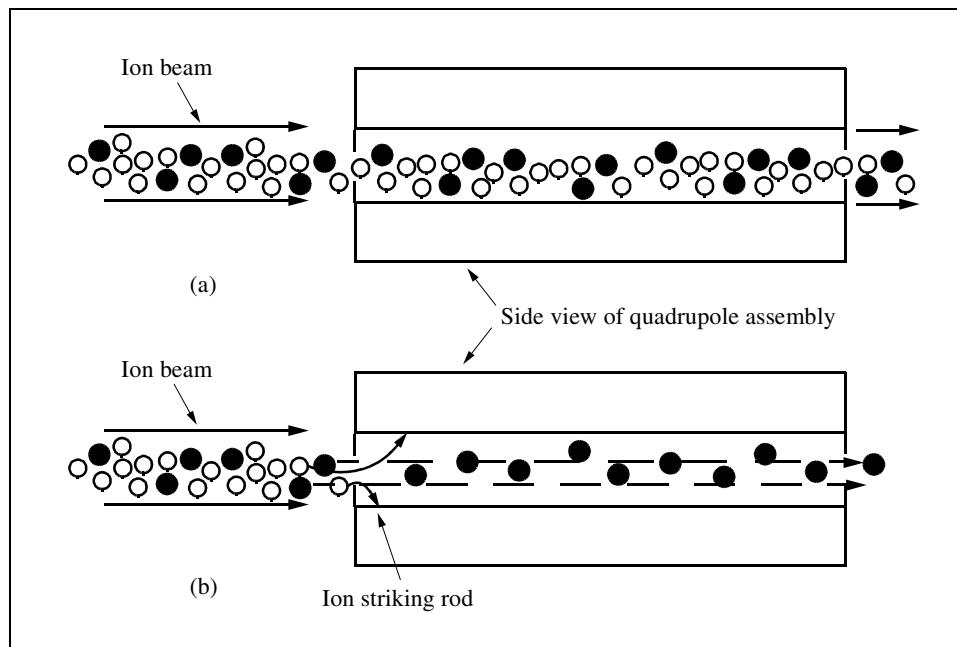


Figure 3 (a) A schematic drawing of ions in a beam entering a quadrupole assembly. The shaded circles represent ions of one particular m/z value that are to be selected. The open circles represent all ions of other m/z values that will not be selected. Diagram (a) represents the wide bandpass mode and all ions (shaded and unshaded) are transmitted.

(b) In the narrow bandpass mode, the shaded ions have been selected and pass through the quadrupole assembly but other ions follow trajectories that lead to them striking the quadrupole rods and being lost; for sake of clarity, only two such incidents are shown.

This effect, when added to that due to doubling in length of the flight time, results in significant improvement in the resolution of the TOF instrument.

Operation of the Hybrid

(i) With the Quadrupole in Wide Bandpass Mode

All ions from an ion source are injected into the quadrupole via a hexapole unit (Figure 1). In the ion source, the ions produced have approximately thermal energies corresponding to a room temperature ground state. On passing through the skimmer, the ions are accelerated to supersonic speeds by the gas expansion that occurs. The first hexapole has a high enough gas pressure to slow down the ions to thermal energies once more. Next, the quadrupole allows all of the ions to pass through in its wide bandpass mode. The ion beam is then collimated by two further hexapoles before passing through an electric lens, which defines the beam and accelerates the ions to a kinetic energy of about 40 volts. The ions reach the pusher electrode and a full mass spectrum is obtained in the TOF analyser. Thus, in this configuration, ions are simply transmitted from an ion source through the quadrupole and into the TOF analyser. The ion source is shown as a solution electrospray inlet in Figure 1.

(ii) With the Quadrupole in Narrow Bandpass Mode

Ions from an ion source are injected into the quadrupole via the hexapole unit as before. Now however, ion selection is made. By adjusting DC and RF voltages on the poles, ions of a particular m/z value are chosen (Figure 3). The middle hexapole (Figure 1) becomes a gas collision cell by increasing the background gas pressure within this assembly (this is the reason the assembly is shown encased in a container having holes just large enough to transmit the ion beam but small enough that it is possible to maintain fairly easily a small gas pressure against the effect of the vacuum pumps). The selected ions emerging from the quadrupole, collide with gas molecules in this hexapole, and gain sufficient internal energy to fragment. Thus, selected precursor ions are caused to dissociate to give product (fragment) ions. The product ions and any unchanged precursor ions travel on to the TOF analyser, in which a mass spectrum is obtained. Thus, the hybrid has been operated in MS/MS precursor ion mode.

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Some Advantages of the Hybrid

Figure 1 shows ions being produced from an electrospray source. Generally, these ions are said to be ‘thermalized’, which is to say that their distribution of internal energies is close to that pertaining to their normal room temperature ground state. Such ions have little or no excess of internal energy and exhibit no tendency to fragment. This is an enormous advantage for obtaining molecular mass information from the stable molecular ions. However, a molecular mass gives relatively little information about how the constituent atoms are associated in the ion or in the original molecule from which it came. There is a lack of structural information. By causing the molecular ions to fragment, product ions are produced, which are characteristic of the structure of the original (precursor) ion and therefore of the original molecule. By being able to obtain an unequivocal relative molecular mass, or even a molecular formula derived from that mass, and then obtaining structural information, the hybrid mass spectrometer becomes a powerful tool for investigating single substances or mixtures of substances.

Other instrumental advantages include its high sensitivity and a linear mass scale to 10,000 at full sensitivity. The linearity of the mass scale means that it is only necessary to calibrate the spectrometer using single or sometimes two known mass standards. Some calibration is necessary because the start of the mass scale is subject to some instrumental ‘zero offset’. The digitized accumulation of spectra provides a better signal-to-noise ratio than can be obtained from one spectrum alone. In MS/MS mode, the good peak shapes obtained for product ions with a resolution of about 500 at half peak height afford excellent mass spectra.

Summary

In one instrument, a hybrid of a quadrupole and a TOF analyser, a full mass spectrum of ions produced in an ion source can be measured. If these are molecular ions, their relative molecular mass is obtained. Alternatively, precursor ions can be selected for MS/MS so as to give a fragment ion spectrum characteristic of the precursor ions chosen, which gives structural information.

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CHAPTER D1

ION OPTICS OF MAGNETIC/ELECTRIC SECTOR MS

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Quick Guide

- Substances are converted into species having positive or negative charges (*ions*) in the ion source.
- For an ion of mass (m) and a number (z) of positive or negative charges, the value m/z is an important mass spectrometric observable.
- A stream of ions (*an ion beam*) is directed out of the ion source towards a collector which records their arrival.
- As with a light beam and glass lenses, an ion beam can be directed and focused using *electric and magnetic fields*, often called lenses by analogy with their optical counterparts.
- The system of electric and magnetic fields or lenses is called the *ion optics* of the mass spectrometer.
- Electric lenses correct aberrations in the shape of the ion beam.
- Electric and magnetic fields can be used sequentially, as described in this issue. Crossed electromagnetic fields are described in the separate issue on quadrupoles.
- Another important property of electric and magnetic fields is their ability to separate ions according to their individual masses ($m_1, m_2 \dots m_n$) or, more strictly, their mass-to-charge ratio ($m_1/z, m_2/z \dots m_n/z$).
- After the ion source, the ion optics split the ion beam into its component m/z values (compare splitting white light into a spectrum of colours).
- By changing the strengths of the electric and magnetic fields, ions of different m/z values can be focused at just one spot (the collector).
- From the strengths of the electric and magnetic fields, m/z values are measured.
- A chart showing the number of ions (abundance) arriving at the collector and their respective m/z values is a *mass spectrum*.

Summary

The ion optics of a mass spectrometer cause the ion beam leaving the ion source to arrive at a collector after being separated into individual m/z values and focused.

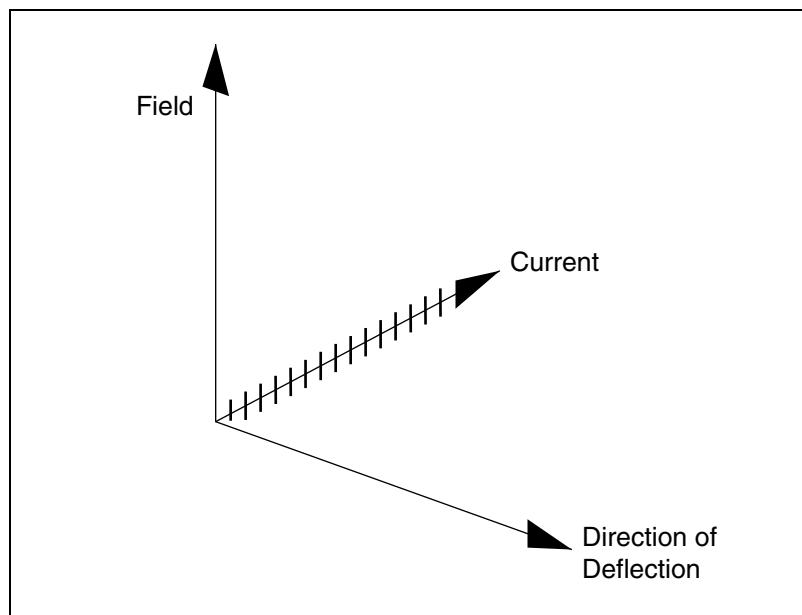


Figure I Fleming's Left Hand Rule

ION OPTICS OF MAGNETIC/ELECTRIC SECTOR MASS SPECTROMETERS

Preamble In the ion source, substances are converted into positive or negative ions having masses (m_1 , m_2 , m_n) and a number (z) of electric charges. From a mass spectrometric viewpoint, the ratio of mass to charge (m_1/z , m_2/z m_n/z) is important. Generally, $z = 1$, in which case, $m_1/z = m_1$, $m_2/z = m_2$ $m_n/z = m_n$, so that the mass spectrometer measures masses of ions. To do this a stream of ions (the ion beam) is injected into the mass analyser region, a series of electric and magnetic fields known as the ion optics. In this region, the ion beam is focused, corrected for aberrations in shape and the individual m/z ratios measured. The ion beam finally arrives at a collector which measures the number (abundance) of ions at each m/z value. The width and shape of the ion beam is controlled by a series of slits (object or source, collector, alpha etc.), situated between the ion source and the collector. A chart of m/z values and their respective abundances makes up the mass spectrum. Ion optics are considered in greater detail below.

Mass Analysis of Ions In this section, magnetic and electric sectors and electric focusing lenses are discussed.

Magnetic Sector When moving charged species (ions) experience a magnetic field, they are deflected. The direction of the deflection can be described by Fleming's 'left hand rule' (Figure 1).

The magnitude of the deflection is governed by the momentum of the ion and is described by the following equations (1,2). Firstly, the kinetic energy of the ion is equal to the energy gained through acceleration from the ion source (equation 1).

$$zV = \frac{1}{2}mv^2 \quad (1)$$

Secondly, the centrifugal force on the ion as its path is deflected by a magnetic field is equal to the force exerted by the field on a moving charge (equation 2).

$$\frac{mv}{r} = zB \quad (2)$$

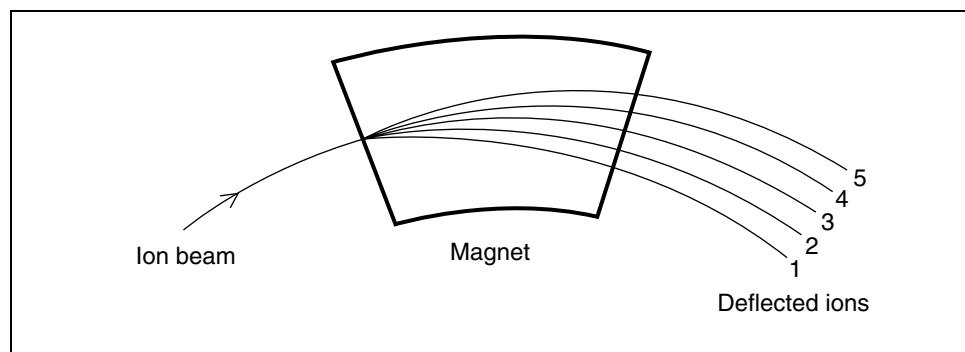


Figure 2 Deflection in a magnetic field of an ion beam consisting of increasing mass-to-charge ratios, $m_1/z, \dots, m_5/z$ and split into different trajectories (1-5) respectively

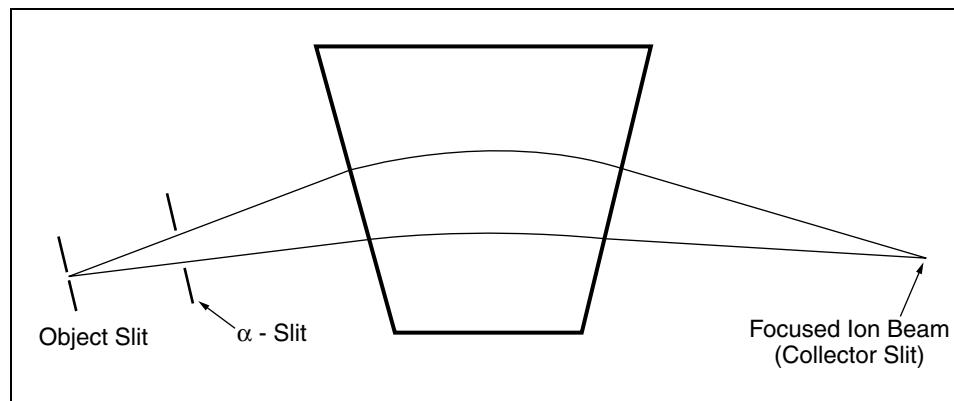


Figure 3 Directional (or angular) focusing of a magnet.

From equations (1,2), the velocity of the ion can be eliminated to give the relationship (3).

$$\frac{m}{z} = \frac{B^2 r^2}{2V} \quad (3)$$

Where:

r = radius of arc of ions being deflected in the magnetic field

V = accelerating potential applied to ions leaving the ion source

B = magnetic field strength

z = number of charges on an ion

m = mass of any one ion

v = velocity of an ion after acceleration through the electric field (V).

If only ions with a single charge ($z = 1$) are considered then, with a constant field strength and constant accelerating voltage, the radius of arc depends on mass and, from (3), equation (4) is obtained.

$$r = \sqrt{\frac{m^2 V}{B^2}} \quad (4)$$

Thus, it is possible to separate ions of different mass (Figure 2) with ions arriving at position 1 (greater deflection) being of lower mass than those arriving at position 5 (lesser deflection). In the modern scanning mass spectrometer, it is more convenient that ions should arrive at a single point for monitoring (collection) and so r (or r^2) is kept constant. This means that B and/or V must be varied to bring all ions to the same focus, viz., one of the relationships (5) must apply:

$$m \propto B^2 \quad (V \text{ constant})$$

$$m \propto 1/V \quad (B \text{ constant})$$

$$m \propto B^2/V \quad (5)$$

From these relationships, (5), it can be seen that, if either the magnetic field (B) or the voltage (V) or both B and V are scanned, the whole range of masses of the ions may be brought into focus sequentially at a given point, the collector. Generally, a scanning magnetic sector mass spectrometer carries out mass analysis by keeping V constant and varying the magnetic field (B).

A further property of the magnetic field is that a diverging ion beam entering that field leaves with the beam converging. Thus, the magnet is said to be directional (or angular) focusing (Figure 3).

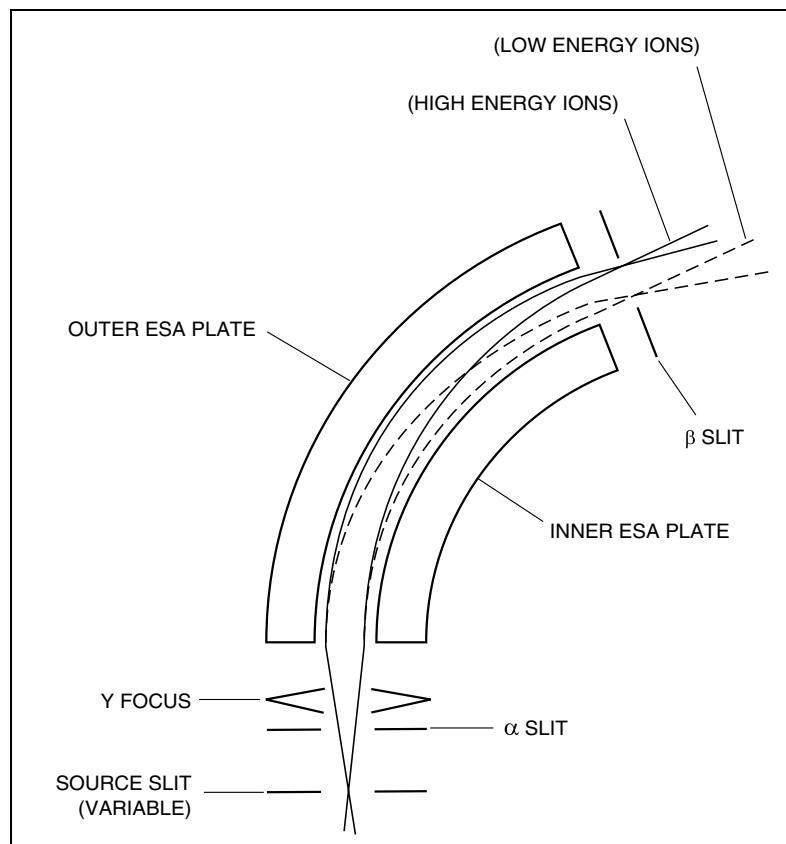


Figure 4 Focusing and dispersion properties of an electrostatic analyser.

So far, it has been assumed that all ions leaving the source have exactly the same kinetic energy but this is not really the case. In EI, the spread in kinetic energy can be as much as 1 volt and, with FAB can be as much as 4 volts. This spread results in a blurred image at the collector because the magnet has no energy focusing and ions of different kinetic energies are brought to slightly different foci. Thus a single magnetic sector has directional (or angular) focusing and therefore is said to be *single focusing* only.

Electrostatic Analyser (Electric Sector)

An electrostatic analyser (ESA) is a directional (or angular) focusing device and is also energy dispersive (Figure 4).

As shown in equations (1,2), the energy gained by ions accelerated from the ion source is $zV\left(=\frac{1}{2}mv^2\right)$ and in the electric sector, the centrifugal force acting on the ions is given by equation (6),

$$zE = \frac{mv^2}{R} \quad (6)$$

where:

E = electric potential (voltage) between the inner and outer ESA plates.

R = radius of curvature of ion trajectory

From these equations, the relationship (7) is obtained.

$$R = \frac{2V}{E} \quad (7)$$

No mass or charge appears in this equation so that, in the electric sector, the ion flight path bends in an arc, which depends only on the accelerating voltage (V) and the ESA voltage (E).

Magnet / Electrostatic Analyser Combination

The ion beam is collimated when a magnetic analyser is combined with an ESA, the combination can be made both energy and mass focused, vis., the ion beam is collimated in the ESA and then properly focused in the magnetic field (Figure 5). The combination is called *double focusing* because it is both directional (or angular) and energy focusing. The double focusing mass spectrometer is designed such that ions of different energies (but of the same mass), converge at the collector (Figure 5).

Double Focusing Forward Geometry ion optics is a combination, in which the ESA is placed before the magnet as shown in Figure 5.

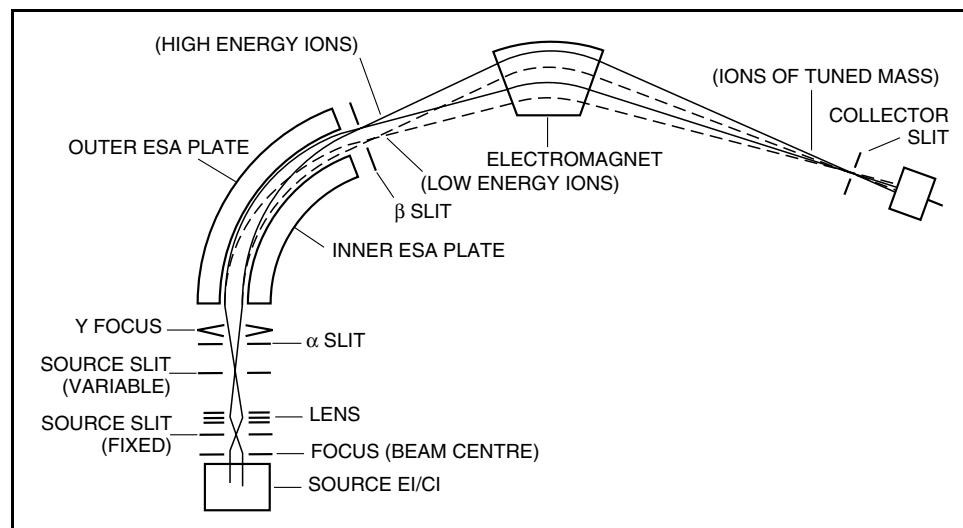


Figure 5 Double focusing ion optics (forward geometry).

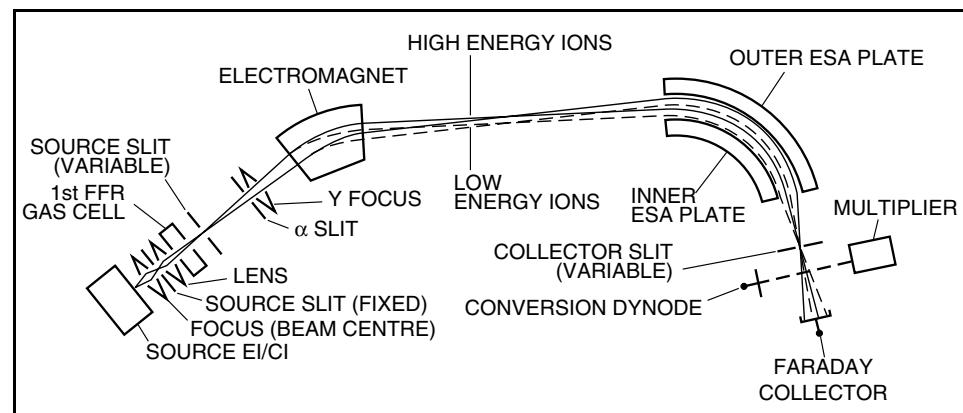


Figure 6 Double focusing ion optics (reverse geometry).

Double Focusing Reverse Geometry ion optics is a combination, in which the magnet is placed before the ESA and is shown in Figure 6.

The double focusing combination of electrostatic and magnetic sector analysers allows the inherent energy spread of the beam to be compensated for by design, and ensures that there is no spread in the beam at the collector arising from either of these sources.

Electric Focusing Lenses

Y-Focus, Z-Focus and Deflect Lenses

It has been stated above that the focus of all masses will occur at a single position, the collector slit (Figures 5, 6). However, because the actual shape of the field within and around the pole tips of the magnet varies with changing field, especially at higher field strengths, the final focal point of the beam shifts as field strength changes. This leads to a change of focus with mass and affects the ability of the instrument to resolve small mass differences. On early mass spectrometers, the problem could be corrected by physically adjusting the position of the magnet for any given mass. On modern instruments, an electric field called the *Y-focus* is used to compensate for these imperfections (Figures 5, 6). The aim of this lens is to focus the ions at the same position (the collector slit) throughout the mass range. Thus, using the electric and magnetic sectors with a *Y-focus* lens ensures all ions are brought to the same focus and allows small differences in mass to be detected, viz., the resolution of the instrument is enhanced.

On VG instruments, these lenses are sited before and after the magnetic sector. The focus and deflection lenses are used to steer the beam so that it coincides with the gap in the collector slit. The *Z-focus* lenses are used to change the divergence of the beam by adjusting voltages on lens plates situated on either side of the beam. The deflection lenses are used to move the whole beam; the *Y-deflection* lens is used to move the beam from one side to the other and the *Z-deflection* moves the beam up or down. The two lenses allow the ion beam to be aligned correctly with the collector slit. Voltages on the lens plates are adjusted to effect such movements of the beam (Figure 7).

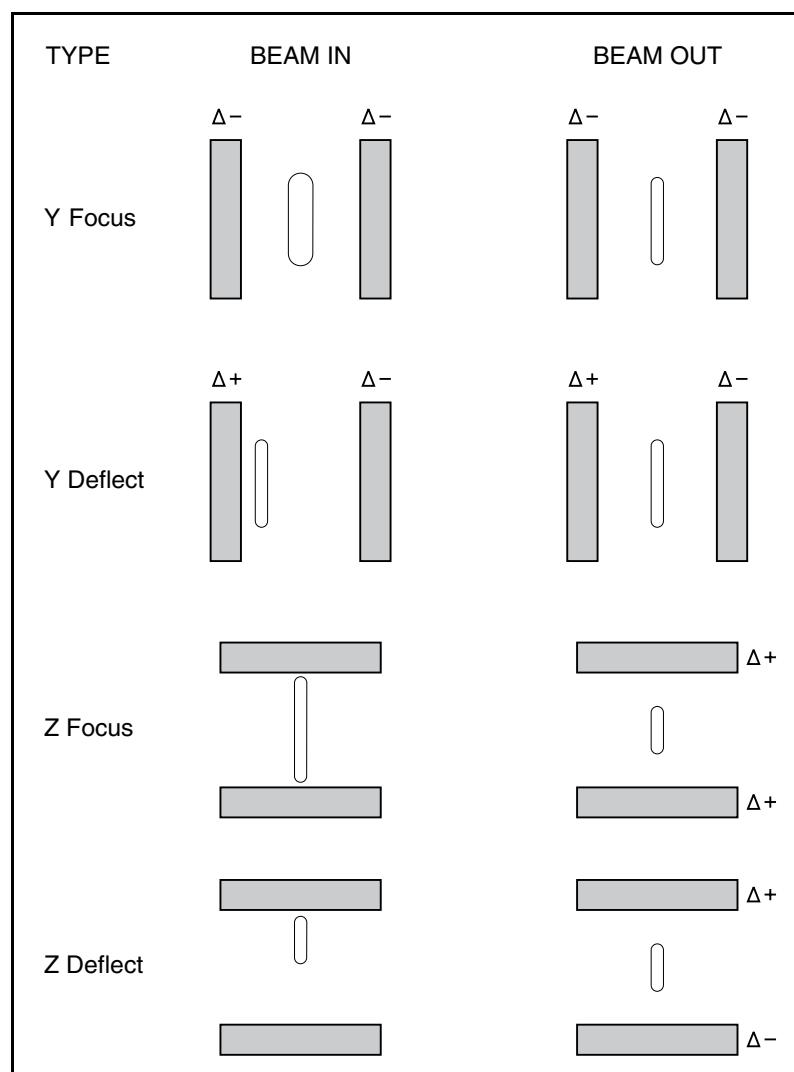


Figure 7 Y-Focus, Z-focus and deflect lenses with their effects on the ion beam

Curvature and Rotation Lenses

Curvature and rotation lenses are used to correct for any imperfections (aberrations) in the cross-sectional shape of the beam before it reaches the collector slit. The *curvature lens* provides a means of changing any ‘banana’ shaped beam cross-section into a rectangular shape (Figure 8). The *rotation lens* is used to rotate the beam such that the sides of the beam become parallel with the long axis of the collector slit (Figure 8).

Metastable Ions

An ion beam mainly comprises ‘normal ions’ all having the same kinetic energy gained on acceleration from the ion source but there are also some ions in the beam with much less than the full kinetic energy; these are called ‘metastable’ ions.

Energy Filter

An *energy filter* is a system of electrostatic fields which strictly has little to do with the main focusing fields but, rather, provides a means of discriminating between normal and metastable ions. The system is a filter, preventing metastable ions from being detected by the collector and it consists of a series of parallel lens plates to which is applied a decelerating voltage of 90-99% of the original accelerating potential (V). Normal ions have enough energy to pass through the filter, to reach the collector but metastable ions do not.

Conclusion

Through the use of sequential electric (electrostatic) and magnetic fields (sectors) and various correcting lenses, the ion beam leaving the ion source can be adjusted so that it arrives at the collector in focus and with a rectangularly shaped cross-section aligned with the collector slits. For the use of crossed electromagnetic fields, the relevant issue on quadrupole instruments should be consulted.

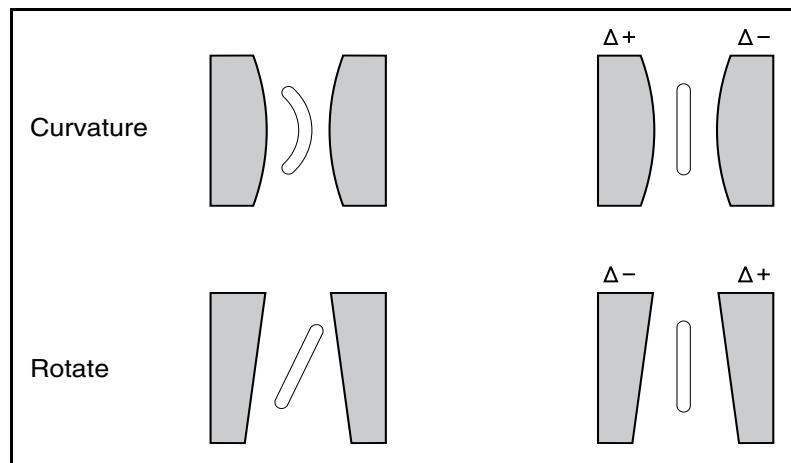


Figure 8 Curvature and Rotate lenses and their effects on the ion beam.

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CHAPTER D2

QUADRUPOLE ION OPTICS

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Quick Guide

- In a quadrupole mass analyser assembly four (quad) parallel rods (poles) are arranged equidistantly from a central (imaginary) axis.
- Static and alternating (radio frequency) electric potentials are applied to opposite pairs of rods, to give a resultant fluctuating electric field.
- Positive or negative ions (electrically charged species) from a source are injected along the central axis of the quadrupole assembly.
- For particular magnitudes and frequencies of the electric fields, only ions of selected mass can pass (filter) through the assembly to reach an ion detector.
- Those ions with masses too large or too small to pass through the quadrupole, strike the rods and are lost.
- The selection of which ions can pass through the quadrupoles to reach the detector is made by varying the electric potentials and/or their frequency; usually it is easier to keep the frequency constant and alter the voltage.
- By altering the electric fields in a consistent manner, the masses of all ions formed in the source can be scanned sequentially from low mass to high or vice-versa to give a mass spectrum.
- The appearance of the mass spectrum is closely similar to that provided by a magnetic sector instrument.

Summary

Through the application of DC and RF voltages to an assembly of four parallel rods, ions can be filtered along their central axis and mass measured to give a mass spectrum.

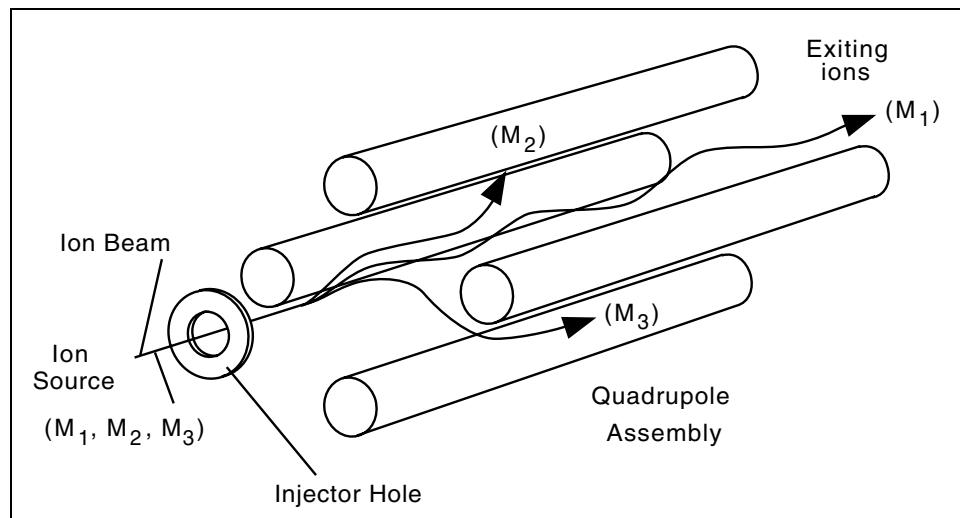


Figure I For a beam of ions injected into the quadrupole assembly from an ion source, then depending on mass, some (M_1) pass along the central axis but others (M_2, M_3) are deflected to the rods.

QUADRUPOLE ION OPTICS

Background

Moving charged species such as ions, passing through magnetic or electric fields (sectors) experience a force which deflects them from their original trajectory. This effect is utilized in magnetic sector mass spectrometers for separating ions according to mass or, strictly, mass-to-charge ratio (m/z), the deflection being related to m/z and magnetic field strength (see ‘Ion Optics’). In a quadrupole instrument, only electric fields are used to separate ions according to mass, as they pass along the central axis of four parallel, equidistant rods (poles) which have fixed (DC) and alternating (RF) voltages applied to them (Figure 1). Depending on field strengths, it can be arranged that only ions of one selected mass can pass (filter) through the rod assembly whilst all others are deflected to strike the rods. By changing the strengths and frequencies of electric fields, different masses can be filtered through the system to produce a *mass spectrum*.

Equations of Motion of Ions

Unlike simple deflections or accelerations of ions in magnetic and electric fields (‘Ion Optics’), the trajectory of an ion in a quadrupolar field is complex and the equations of motion are less easy to understand. Accordingly, a simplified version of the equations is given here with a fuller discussion in the Appendix.

The four rods of circular cross-section shown in Figure 1 should theoretically each have a hyperbolic cross-section (Figure 2a) but, in practice, cylindrical rods (Figure 2b) are quite satisfactory if properly spaced apart. Two opposed rods have a potential of $+(U + V\cos\omega t)$ and the other two, $-(U + V\cos\omega t)$ where U is a fixed potential and $V \cos(\omega t)$ represents a radio frequency (RF) field of amplitude (V) and frequency (ω). Thus, as $\cos(\omega t)$ cycles with time (t), the applied voltages on opposed pairs of rods (A, B) change in the manner shown in Figure 3. Along the central axis of the quadrupole assembly and along the two planes shown in Figure 2b, the resultant electric field is zero.

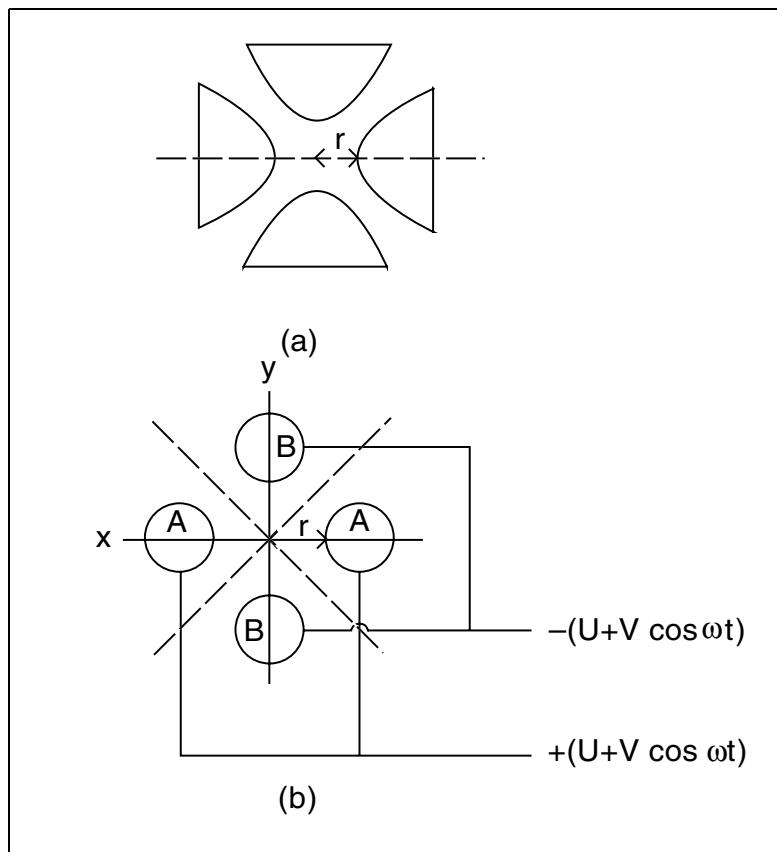


Figure 2 End views of the quadrupole assembly (a) showing the theoretically desired cross-section and (b) illustrating the practically easier to obtain system. In (b), a positive potential, $+(U + V \cos \omega t)$, is applied to two opposed rods (A) and a negative potential, $-(U + V \cos \omega t)$ to the other two (B). The dotted lines indicate planes of zero electric field. The dimension (r) is typically about 5 mm with rod diameters of 12 mm. The x- and y- axes are indicated with the z axis being perpendicular to the plane of the paper.

In the transverse direction of the quadrupoles, an ion will oscillate amongst the poles in a complex fashion, depending on its mass, the voltages (U , V) and the frequency (ω) of the alternating RF potential. By suitable choices of U , V and ω , it can be arranged that only ions of one mass will oscillate stably about the central axis; in this case all other ions will oscillate with greater and greater amplitude until eventually they strike one of the poles and are lost (unstable motion). Unlike this transverse situation, in the direction of the central axis of the quadrupole assembly, the applied field is zero and an ion trapped in the quadrupolar field will not pass through the assembly unless it has first been given some momentum (a push) in that direction. To give ions this forward momentum, they are accelerated through a potential of about 5 volts, maintained between the ion source and the quadrupole. Usually, this injected ion beam is divergent and in order to reduce the spread in the beam and improve mass resolution it is passed through a small defining hole or aperture placed between the source and the quadrupole (Figure 1).

Therefore, ions passing into the quadrupole assembly move along the central axis with a complex oscillatory trajectory, those with the 'right' values of m/z having a stable trajectory and being able to pass right through to the detector but those with the 'wrong' values having unstable trajectories and being lost to the poles. By suitable variation of U , V and ω , ions of different masses can pass through the quadrupole filter successively and be detected. The numbers (abundances) of ions arriving at the detector for each m/z value give the mass spectrum in the usual way.

In practice, the frequency (ω) is fixed, typical values being in the range 1-2 MHz. The DC voltage (U) may be 1000 volts and the maximum RF voltage (V), 6000 volts. The pole assemblies range in length from about 50 to 250 mm, depending on application and pole diameters lie in the range of 6 to 15 mm. Very high mechanical accuracy to the micron level in both the rods and their spacing is needed for high performance. For example, with a spacing (r) accurate to $10^{-3}r$, the maximum resolution would be about 500 ($0.5r/10^{-3}r$). Scanning speeds of up to 6000 amu/sec are routine with quadrupoles and unit mass resolution up to 2000 amu is attainable.

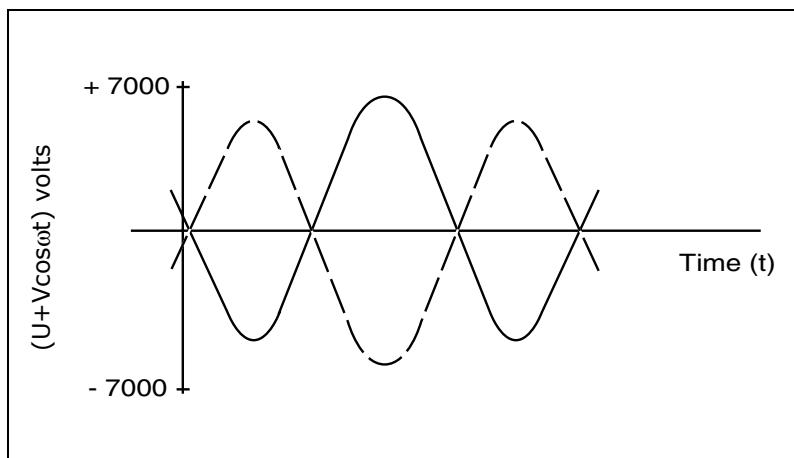


Figure 3 Variation of total voltages with time on each pair of rods A (—) and B (- - -). $U = 1000$ V, $V = 6000$ V and RF is ω .

Comparison of Quadrupole and Magnetic Sector Instruments

Major advantages of quadrupole compared with magnetic instruments include their relatively small size of mass analyser for comparable resolving power, linear mass scale, fast scanning, simplicity of construction, robustness and ease of cleaning. The main disadvantage of the quadrupole lies in ultimate attainable resolving power. As the ratio of the voltage (U,V) changes, a limiting resolution (the ability of the spectrometer to separate two adjacent masses) is reached largely determined by the accuracy with which the various quadrupole parameters are controlled and by the initial motion of the ion as it enters the quadrupole field. A second, less important disadvantage is that the efficiency of transmission of ions through the quadrupole decreases as ion mass increases, leading to a relative loss in sensitivity at higher masses.

Thus, where magnetic sector and quadrupole instruments might be considered for a particular application, ultimate resolving power could be a decisive factor. For example, for use in a gas chromatograph/mass spectrometer combination (GC/MS), because most compounds which are volatile enough to pass through the GC will have relative molecular masses of well under 800, the lower ultimate resolving power of the quadrupole is less important and its lower cost becomes decidedly advantageous.

There are other characteristics of quadrupoles which make them cheaper for attainment of certain objectives. As examples, quadrupoles can easily scan a mass spectrum extremely quickly and are useful for following fast reactions; the quadrupole does not operate at the high voltages used for magnetic sector instruments and coupling to atmospheric pressure inlet systems becomes that much easier because electrical 'arcing' is much less of a problem.

The Choice of Quadrupole or Magnetic Sector Instruments

Modern mass spectrometers are used in a very wide variety of situations so that it becomes almost impossible to have a simple set of criteria that would determine whether a quadrupole or magnetic sector instrument could be best for any particular application. Nevertheless, some attempt is made here to address major considerations, mostly relating to cost.

Table 1 Some Factors Important in Choosing between Quadrupole and Magnetic Sector Mass Spectrometers

Factor	Quadrupole	Magnetic
(i) Overall Cost	+++	+
(ii) Ease of use	+++	+
(iii) Fast scanning or peak switching	++	+
(iv) Ease of coupling to inlet systems	++	+
(v) Ultimate resolving power (accurate mass determination)	+	+++
(vi) Ultimate mass range (over about 4000 a.m.u.)	+	+++
(vii) Ultimate sensitivity, particularly at high mass.	+	+++

The use of the plus (+) signs is meant only as a qualitative guide and should not be construed as a definitive assessment. The greater the number of plus signs, the more advantageous the system.

The most important deciding factor is undoubtedly the end use of the instrument - is it to be used for a very wide range of general applications or for a narrow specialized area of work? Generally, a magnetic sector instrument is capable of ultimate higher resolution and accuracy of mass measurement. Other considerations, some of which are shown in the Table 1, give the advantage to the quadrupole. It should be noted that, in mass spectrometry/mass spectrometry (MS/MS) applications, quadrupole and magnetic sectors can be used together advantageously.

Conclusion

By injecting ions along the central axis of four parallel, equidistantly-spaced rods (poles) to which are applied variable static (DC) and alternating (RF) electric fields, ions can be separated according to mass. The distance apart of the rods, and the magnitudes and frequencies of the applied electric voltages are all important in determining which ion masses traverse the assembly and which instead, strike the rods.

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Appendix

More Details of Equations of Motion

Under the influence of the varying electric fields, $+(U + V\cos\omega t)$ and $-(U + V\cos\omega t)$, the resultant electric potential (F) in the xy plane (transverse direction) of the quadrupoles is given by equation (1).

$$F = \frac{x^2 - y^2}{r^2} (U + V\cos\omega t) \quad (1)$$

Thus, for $x = y$, $F = 0$ and this gives rise to planes of zero field strength (Figure 2b).

At all other positions between the poles, the oscillating electric field (F) causes ions to be alternately attracted to and repelled by the pairs of rods (A, B; Figure 2). Note that equation (1) shows that the field (F) has no effect along the direction of the central (z -) axis of the quadrupole assembly and so, to make ions move in this direction, they must first be accelerated through an electric potential (typically 5 volts) between the ion source and the assembly.

Because of the oscillatory nature of the field (F ; Figure 3), an ion trajectory as it moves through the quadrupole assembly is also oscillatory. For the 'right' values of the parameters U, V, ω and m/z , an ion can pass right through the length of the assembly but, with the 'wrong' values, the field (F) deflects an ion such that it strikes one of the four rods and is lost (Figure 1). Thus, for any particular m/z value, the passage of an ion through the quadrupole depends critically on the DC voltage (U), the RF amplitude (V), the RF frequency (ω) and the distance apart of the poles (r ; Figure 2).

Passage through the quadrupole assembly is described as stable motion, whilst those trajectories which lead to ions striking the poles is called unstable motion.

From mathematical solutions to the equations of motion for the ions, based on equation (1) two factors (a, q ; equation 2) emerge as being important in defining regions of stable ion trajectory.

$$a = \frac{8zU}{mr^2\omega^2} ; \quad q = \frac{4zV}{mr^2\omega^2} ; \quad \frac{a}{q} = \frac{2U}{V} \quad (2)$$

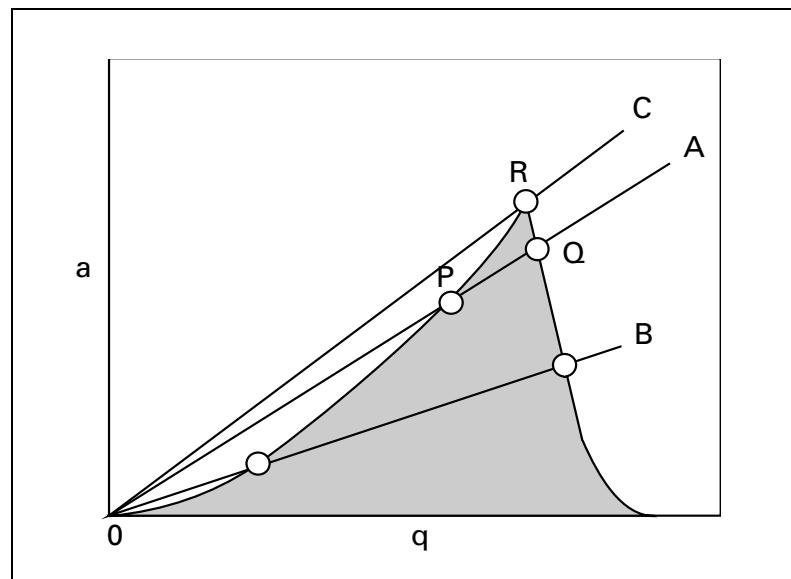


Figure 4 Relationship between a and q . The shaded area indicates regions of stable ion motion through the quadrupolar field.

For small values of a and q , the shaded area in Figure (4) indicates an area of stable ion motion, viz., it shows all values for a,q for which ions can be transmitted through the quadrupole assembly.

To gain some idea of the meaning of this shaded area, consider the straight line OA of slope (a/q) shown in Figure (4). The line enters the region of stable motion at P and leaves it at Q. For typical values of U (1000 volts), V (6000 volts), ω (1.5 MHz) and r (1.0), equations (2) predict that point P corresponds to an ion of m/z 451 and Q to m/z 392. Therefore, with these parameter values, all ions having m/z between 392 and 451 will be transmitted through the quadrupole.

For a line (OB; Figure 4) of smaller slope (smaller a/q or smaller U/V), an even greater range of m/z values will be transmitted through the quadrupole assembly. Conversely, for a line OC which passes through the apex (R) of the region of stability, no ions of any m/z value are transmitted.

To ensure that only ions of any one selected m/z are transmitted (maximum resolution), the parameters (U, V, ω) must be chosen such that a/q (or $2U/V$) fits a line which passes close to R but which still lies within the region of stability. This will give maximum resolution for the instrument. For example, with U (1000 volts), V (6000 volts), ω (2 MHz) and r (1.0 cm), only ions of m/z 862 would be transmitted. To transmit ions of other m/z , the parameters U, V, ω and r have to be changed.

For a given assembly, r is fixed and electronically, it is easier to change voltages (U, V) than frequencies (ω). Therefore, to transmit ions of other m/z values, the frequency is kept constant but the voltages (U, V) are varied in such a way that U/V (or a/q) remains constant. By continuously increasing or decreasing U and V whilst keeping U/V constant, ions of increasing or decreasing m/z successively traverse the quadrupole assembly to give a mass spectrum.

For convenience, different frequencies may be used for different mass ranges, e.g. the RF may be 1.5 MHz for 0 - 1000 amu and 0.8 MHz for 1000 - 4000 amu.

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CHAPTER D3

TOF ION OPTICS

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Quick Guide

- In a *Time-Of-Flight* (TOF) mass spectrometer, ions formed in an ion source are extracted and accelerated to a high velocity by an electric field into an analyser consisting of a long straight 'drift tube'. The ions pass along the tube until they reach a detector.
- After the initial acceleration phase, the velocity reached by an ion is inversely proportional to its mass (strictly, inversely proportional to the square root of its m/z value).
- Since the distance from the source to the detector is fixed, the time taken for an ion to traverse the analyser in a straight line is proportional to its velocity and hence its mass (strictly, proportional to the square root of its m/z value). Thus, each m/z value has its characteristic time-of-flight from the source to the detector.
- In effect, the ions race each other along the drift tube but the winners are always the ions of smallest m/z value since these have the shortest flight times. The last to arrive at the detector are always those of greatest mass which have the longest flight times. However, as in a race, for there to be a separation at the finish line (the detector), the ions must all start from the ion source at the same time (no handicapping allowed!).
- The times taken for ions of differing m/z values to reach the detector are of the order of a few microseconds and the separation in times of arrival for ions of differing m/z value is less than this. Thus, if ions of different mass are to be separated adequately in a time domain (good resolution), they should all start from the ion source at exactly the same time or, more practically, within a few nanoseconds of each other.
- Ions for TOF mass spectrometry must be extracted from the ion source in 'instantaneous' pulses. Therefore, either ions are produced continuously but are extracted from the source in pulses or ions are produced *directly* in pulses.
- TOF mass spectrometry is ideally suited to those ionization methods that inherently produce ions in pulses, as with pulsed laser desorption or Cf-radionuclide ionization.

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- There is no *theoretical* upper limit on m/z that can be examined and TOF mass spectrometry is useful for substances having very high molecular mass. In practice, the current upper limit is about 350,000.
- Unfortunately, ions even of the same m/z value do have a spread of velocities after acceleration and therefore the resolution achievable with TOF is not very high because bunches of ions of one m/z value overlap those at the next m/z value.
- By use of an electrostatic ‘ion mirror’, called a *reflectron* the velocities of ions of the same m/z value can be made more nearly equal, thereby making their arrival times at the detector more nearly equal and so improving resolution.
- After reflection in the reflectron, the ions must pass down a second length of analyser set at a small angle to the first so as to reach the detector.
- The improvement in resolution with the reflectron is achieved at the expense of some loss in overall sensitivity due to loss of ions in the reflectron and in the second length of analyser.
- For very high mass, when sensitivity is frequently critical, the reflectron is not used and lower resolution is accepted.
- The mass spectrum gives the abundances of ions for different times of arrival at the detector. Since the times are proportional to the square root of the m/z values, it is simple to convert the arrival times into m/z values.

Summary	After acceleration through an electric field, ions pass ('drift') along a straight length of analyser under vacuum and reach a detector after a time which depends on the square root of their m/z values. The mass spectrum is a record of the abundances of ions and the times (converted to m/z) they have taken to traverse the analyser. TOF mass spectrometry is valuable for its fast response time, especially for substances of high mass which have been ionized in pulses.
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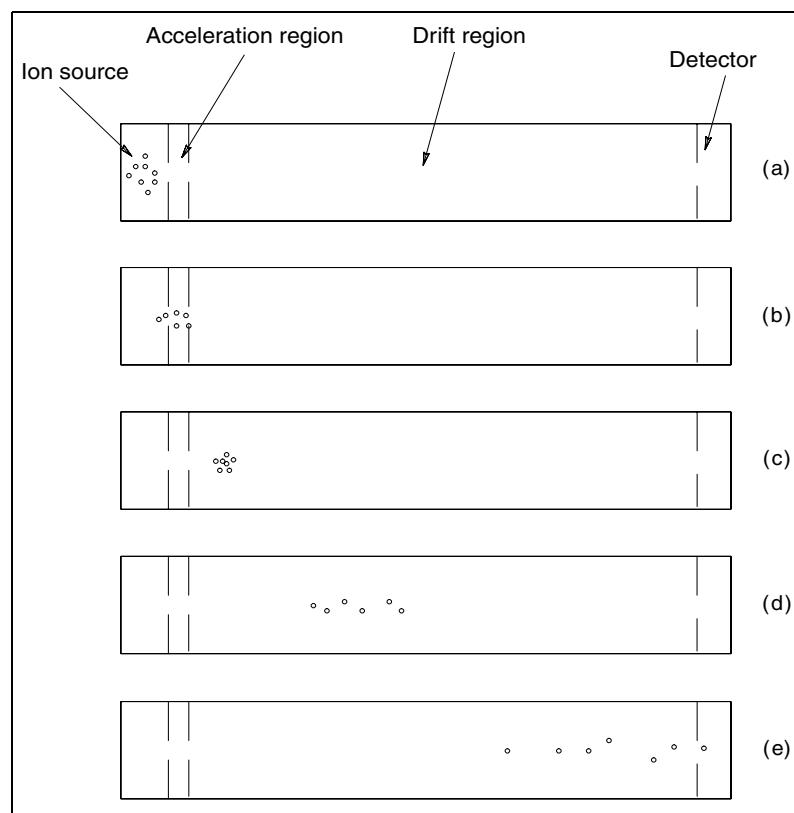


Figure I The essentials of time-of-flight optics. In (a), a pulse of ions is formed and then accelerated out of the source (b) into the drift region (c). After a short time (d), the ions have separated along the drift region according to m/z value. In (e), the ions with smallest m/z value (fastest moving) begin arriving first at the detector, to be followed by the ions of gradually increasing m/z .

TIME-OF-FLIGHT ION OPTICS

Background

Whereas understanding the fundamentals of the ion optics for magnetic and electric sector, quadrupole, ion trap and FT ion cyclotron resonance mass spectrometers ranges from fairly simple to difficult, the basic ion optics of Time-Of-Flight (TOF) instruments is very straightforward. Basically, ions are extracted from the ion source in short pulses and directed down an evacuated straight tube to a detector. The time taken to travel the length of the 'drift' or 'flight' tube depends on the mass of the ion and its charge. For singly charged ions ($z = 1$; $m/z = m$), the time taken to traverse the distance from the source to the detector is proportional to a function of mass, the greater the mass of the ion the slower it is in arriving at the detector. Thus, there are no electric or magnetic fields to constrain the ions into curved or complicated trajectories. After initial acceleration, the ions pass in a straight line, at constant speed, to the detector. The arrival of the ions at the detector is recorded in the usual way as a trace of ion abundance against time of arrival, the latter being converted into a mass scale to give the final mass spectrum.

Equations of Motion of Ions

A short pulse of ions is extracted from the ion source (Figure 1). It is necessary to use a pulse because, using only time to differentiate amongst the masses, it is important that the ions all leave the ion source at the same instant (like runners in a race on hearing a starting pistol). The first step is acceleration through an electric field (E volts). With the usual nomenclature (m = mass, z = number of charges on an ion, e = the charge on an electron, v = the final velocity reached on acceleration), the kinetic energy ($mv^2/2$) of the ion is given by equation (1).

$$mv^2/2 = z.e.E \quad (1)$$

Equation (2) follows by simple rearrangement.

$$v = \sqrt{2z.e.E/m} \quad (2)$$

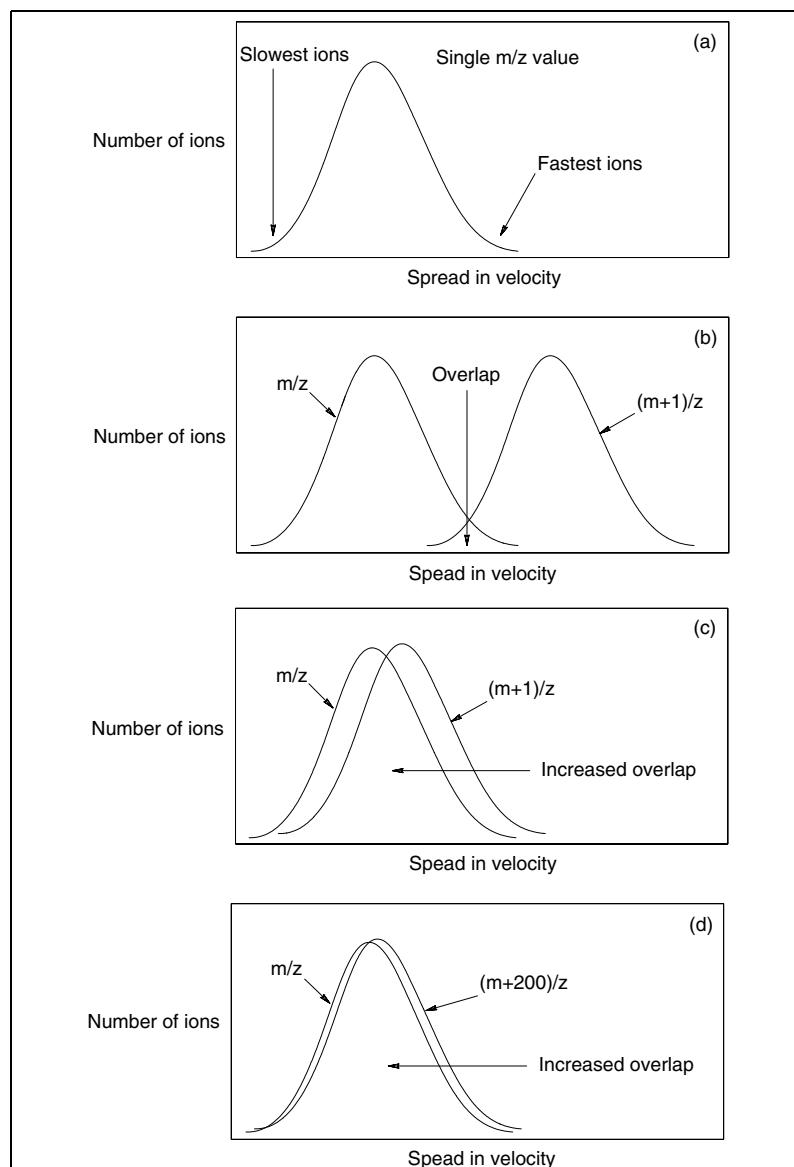


Figure 2 (a) Each set of ions at any one m/z value will have a small spread in speed because they are not all formed at exactly the same place in the ion source. (b) Two sets of ions separated by one mass unit (m , $m+1$) overlap because the slower ions of smaller mass overlap the faster ions of greater mass. (c) For larger m/z values, this effect leads to almost total disappearance of unit resolution. (d) At still greater m/z values, even mass differences of 200 or more may not be separated.

If the distance from the ion source to the detector is d , then the time (t) taken for an ion to traverse the drift tube is given by equation (3).

$$t = d/v = d/\sqrt{2z.e.E/m} = d.[\sqrt{(m/z)}/\sqrt{2e.E}] \quad (3)$$

In equation (3), d is fixed, E is held constant in the instrument and e is a universal constant. Thus, the flight time of an ion t is directly proportional to the square root of m/z (equation 4).

$$t = \sqrt{(m/z)} \times \text{a constant} \quad (4)$$

Equation (4) shows that an ion of m/z 100 will take twice as long to reach the detector as an ion of m/z 25:

$$(t_{100}/t_{25} = \sqrt{100}/\sqrt{25} = 10/5 = 2)$$

Resolution

Generally, the attainable resolving power of a TOF instrument is limited, particularly at higher mass. There are two major reasons for this, one inherent in the technique, the other a practical problem. First, the flight times are proportional to the square root of m/z . The difference in the flight times (t_m and t_{m+1}) for two ions separated by unit mass is given by equation (5).

$$t_m - t_{m+1} = \Delta t = [(\sqrt{m/z}) - (\sqrt{(m+1)/z})] \times \text{a constant} \quad (5)$$

As m increases, Δt becomes progressively smaller (compare the difference between the square roots of 1 and 2 (= 0.4) with the difference between 100 and 101 (= 0.05). Thus, the difference in arrival times of ions arriving at the detector become smaller and smaller and more difficult to differentiate. This inherent problem is a severe restriction even without the second difficulty which is due to the fact that not all ions of any one given m/z value reach the same velocity after acceleration nor are they all formed at exactly the same point in the ion source. Therefore, even for any one m/z value, the ions reach the detector over an interval of time instead of all at one time. Clearly, where separation of flight times is very short, as with TOF instruments, the spread for individual ion m/z values means there will be overlap in arrival times between ions of closely similar m/z values. This effect (Figure 2) decreases available (theoretical) resolution but it can be ameliorated by modifying the instrument to include a reflectron (see below).

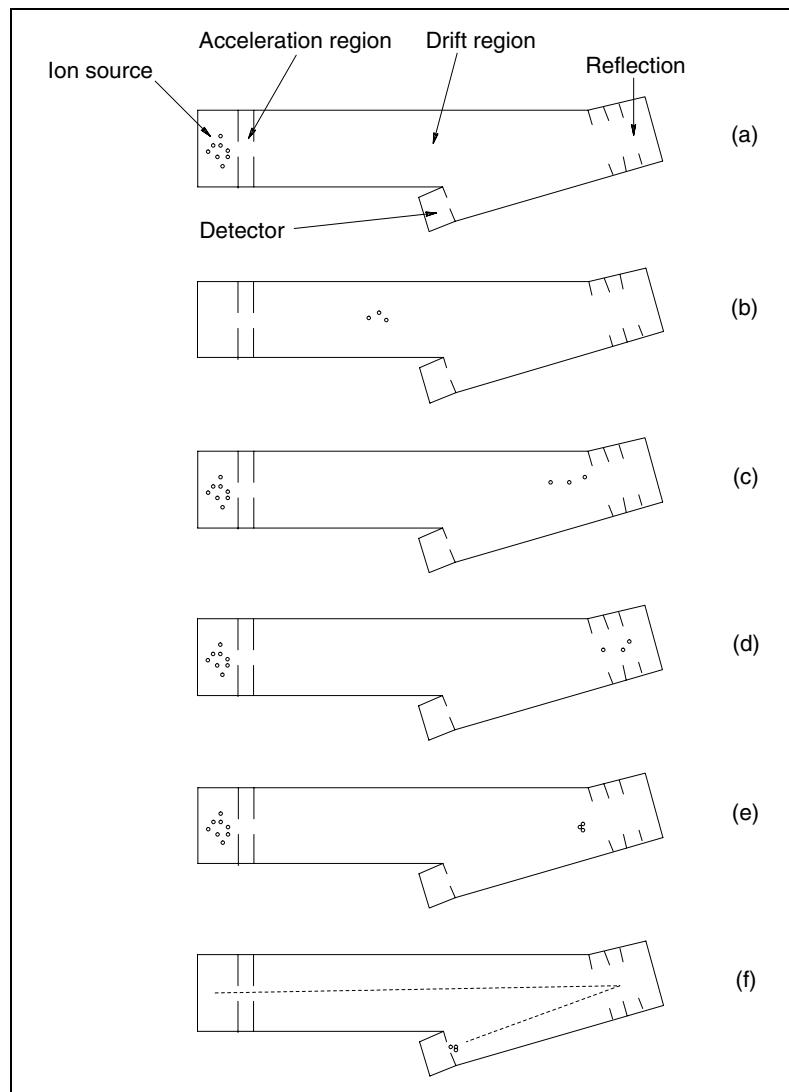


Figure 3 In (a), a pulse of ions is formed but, for illustration purposes, all with the same m/z value. In (b), the ions have been accelerated but, because they were not all formed in the same space, they are separated in time and velocity, some ions having more kinetic energy than others. In (c), the ions approach the 'ion mirror' or reflectron which they then penetrate to different depths, depending on their kinetic energies (d). The ones with greater kinetic energy penetrate furthest. In (e), the ions leave the reflectron in a bunch (i.e., no longer separated in time) and travel on to the detector (f). The path taken by the ions is indicated by the dotted line in (f).

Reflectron

A homogeneous electrostatic field is placed at the end of the flight path of the ions and has a polarity the same as that of the ions, viz., positive (negative) ions experience a retarding positive (negative) potential. The ions come to a stop and are then accelerated in the opposite direction. The ions are reflected by the 'ion mirror' or reflectron. The ion mirror is often at a slight angle (Figure 3) to the line of flight of the ions and, when reflected, the ions do not travel back along the same path but along a slightly deflected line (some instruments reflect ions back along the path they took to the reflection). Consider ions of any one given m/z value. The faster ions, having greater kinetic energy, travel further into the electrostatic field before being reflected than do slower ions. As a result, the faster ions spend slightly more time within the reflectron than do the slower ones. This causes the faster and slower ions to bunch up so that they leave the reflectron closer together and travel onto the detector, where the ions arrive close together. There is a similar effect for all other individual m/z values and overall resolution is greatly improved. Instead of a typical TOF resolution of about 1000, resolutions of around 10,000 can be achieved. There is a disadvantage in using a reflectron in that the sensitivity of the instrument is decreased through ion loss by collision and dispersion from the main beam. It is a more severe problem for ions of large mass and, for these, the reflectron is often not used despite the better resolution attainable with its use

Comparison with other mass spectrometers

TOF mass spectrometers are very robust and usable with a wide variety of ion sources and inlet systems. Having only simple electrostatic and no magnetic fields, their construction, maintenance and calibration are usually straightforward. There is no upper theoretical mass limitation; all ions can be made to proceed from source to detector. In practice, there is a mass limitation in that it becomes increasingly difficult to discriminate between times of arrival at the detector as the m/z value becomes large. This effect coupled with the spread in arrival times for any one m/z value means that discrimination between unit masses becomes difficult at say m/z 3000. At m/z 50,000 overlap of 50 mass units is more typical, i.e., mass accuracy is no better than about 50-100 mass units (Figure 2). Nevertheless, the ability of a TOF instrument to measure routinely and simply masses this large gives it a decided advantage over many other types of mass analyser.

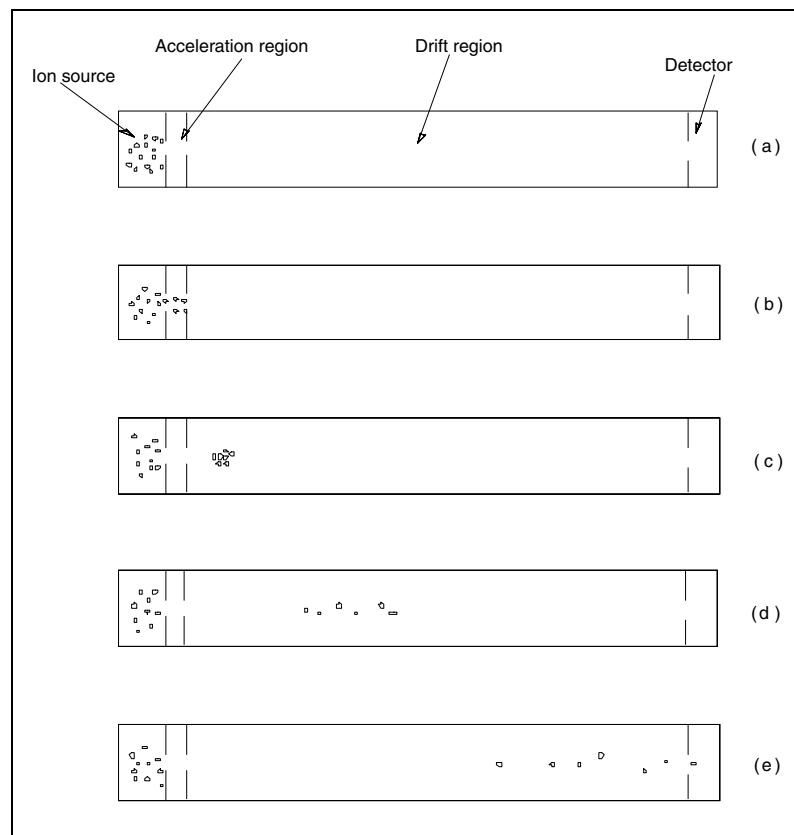


Figure 4 In this mode, ions are formed continuously in the ion source (a) but the electrostatic accelerating potential is applied in pulses (b). Thus, a sample of ions is drawn into the drift region (c) with more ions being formed in the source. As shown in figure 1, the ions separate according to m/z values (d) and arrive at the detector (e), the ions of largest m/z arriving last.

A major advantage of the TOF mass spectrometer is its fast response time and its applicability to ionization methods that produce ions in pulses. As discussed earlier, because all ions follow the same path, all ions need to leave the ion source at the same time if there is to be no overlap between m/z values at the detector. In turn, this means that, if ions are produced continuously as in a typical electron ionization source, then samples of these ions must be utilized in pulses by switching the ion extraction field on and off very quickly (Figure 4). On the other hand, there are some ionization techniques that are very useful, particularly at very high mass, but only produce ions in pulses. For these sources, the ion extraction field can be left on continuously. Two prominent examples are Californium radionuclide and laser desorption ionization. In the former, nuclear disintegration occurs within a very short time frame to give a short pulse of ions; the same disintegration is used to start the timer ('stopwatch') for the race of the ions down the flight tube. Similarly, a laser pulse lasting only a few nanoseconds produces a bunch of ions and acts to start the timer also.

Conclusion

By accelerating a pulse of ions into a flight or drift tube, the ions achieve different velocities depending on their individual m/z values; the bigger the m/z value, the slower the ion travels down the tube. A detector at the end of the tube records the times of arrival of the ions and hence their flight times. The flight times are easily converted into m/z information by a simple formula. The Time-of-Flight mass spectrometer is particularly useful for ions produced in pulses and where fast response times are needed. It can be used to detect ions of very large m/z values.

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Back to Basics Section D: Ion Optics

CHAPTER D4

ORTHOGONAL TIME OF FLIGHT OPTICS

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Quick Guide

- Orthogonal TOF is the name commonly given to orthogonally accelerated Time-of-Flight mass spectrometry. It is sometimes referred to by the acronym oaTOF, especially in official publications, but it is more usual for it to be referred to simply as orthogonal TOF; this abbreviation is used here.
- In this Quick Guide, and purely for the purposes of illustration, orthogonal TOF optics are compared with those from magnetic sector instruments. Greater details of orthogonal TOF analysers hybridized with other kinds of ion optics are given in the relevant sections of Back-to-Basics
- In conventional mass spectrometry with electric and magnetic sectors arranged in-line (see Back-to-Basics, Ion Optics), an ion beam consists of a stream of ions of all m/z values, which is separated into individual m/z values by the magnetic sector before being collected by single-point or multipoint detectors (see Back-to-Basics, Point Ion Collectors and Array Collectors).
- In time-of-flight mass spectrometry, ions of different m/z values are detected as a function of their velocities along a flight tube (see Back-to-Basics, Time of Flight Instruments).
- Thus, it can be said that conventional magnetic sectors separate ions into individual m/z values by dispersion in space (spatially) and not according to their flight times. Contrarily, TOF analysers separate ions of different m/z values according to their velocities (temporally) but not spatially.
- These two types of analyser are frequently used alone but can be used in tandem, with ions from a first magnetic analyser passing through a region, in which there is applied an electric field at right angles to the direction of the ion beam. This orthogonal electric field is pulsed at very short time intervals.
- Ions accelerated from the first analyser have a velocity, which is proportional to the initial accelerating voltage in the ion source. On reaching the ‘orthogonal zone’, the pulsed electric field gives these ions a further velocity but now in a direction at right angles to their original velocity. The resultant velocity is given by the vector sum of the initial and second velocities.

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- After the pulsed electric field has been applied, a pulse of ions is directed into a TOF analyser placed at an angle to the original ion beam. When the pulse is off, the ions have only their original velocities and continue into a different ion collector.
- The pulsed ions start their journeys down the TOF flight tube all at the same time; they separate in the TOF analyser according to their velocities and arrive at the TOF ion collector at different times (temporally separated).
- Therefore the orthogonal TOF mass spectrum is a ‘snapshot’ of all the ions in the ‘sampled’ ion beam at any one moment in time. The arrangement has advantages over either magnetic sectors alone or TOF instruments alone (see Back-to-Basics, Orthogonal TOF Hybrid Instruments, for further discussion).
- An orthogonal acceleration time-of-flight mass spectrometer can be used with ‘continuous’ ion sources with a high sampling efficiency (typically 20-30%). Consequently the orthogonal TOF has a much higher duty cycle than a scanning instrument, which may have a duty cycle of only 0.1-1% when used to record a mass spectrum. This means that the sensitivity will be much higher for the orthogonal TOF mass spectrometer.
- Pulses of ions can be directed into the TOF analyser at the rate of about 30 KHz and therefore, more than 30,000 spectra per second can be collected and summed. There are significant improvements in signal-to-noise ratios and speed of acquisition of data.

Summary

In combined sector/TOF analysers a beam of ions accelerated from an ion source by an electric field and sent into a sector instrument is further subjected to a second pulsed electric field applied at right angles to its initial direction. The resultant pulse of ions sets off along the flight tube of a TOF analyser, where the ions separate into m/z values and are recorded (along with their respective abundances) as a mass spectrum. The combined sector/TOF analysers have several significant advantages, not least for MS/MS studies and improved signal-to-noise ratios.

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ORTHOGONAL TIME-OF-FLIGHT OPTICS

Introduction

Ions produced in an ion source may be separated into their m/z values by a variety of analysers. The resultant set of m/z values, along with the numbers (abundances) of ions forms the mass spectrum. The separation of ions into their individual m/z values has been effected by analysers utilizing magnetic fields or RF electric fields. For example, the mass analysis of ions by instruments using a magnetic field is well-known, as are instruments having quadrupole RF electric fields (quadrupole, ion trap). Ions may also be dispersed in time, so that their m/z values are measured according to their flight times in a time-of-flight instrument. These individual pieces of equipment have their own characteristics and are commonly used in mass spectrometry. In addition, combinations of sectors have given rise to hybrid instruments. The earliest of these was the double-focusing mass spectrometer having an electric sector to focus ions according to their energies and then a magnetic sector to separate the individual m/z values. There is now a whole series of hybrid types, each with some advantage over non-hybrids. Ion collectors have seen a similar improvement in performance and any of the above analysers may be used with ion detectors based on single electron multipliers or in the case of magnetic sectors, on arrays of multipliers or, in the case of ICR, on electric field frequencies. Thus, there is a bewildering variety of instruments potentially available. However, except for very highly specialized purposes, most of the possible hybrids are not used in general mass spectrometry and only a few types are in common use. One of these is the so-called orthogonal TOF instrument. The purpose of the present Back-to-Basics guide is to describe the orthogonal arrangement and to discuss some of its advantages. Actual operation of hybrid orthogonal TOF instruments is discussed under their own headings in other sections of Back-to-Basics.

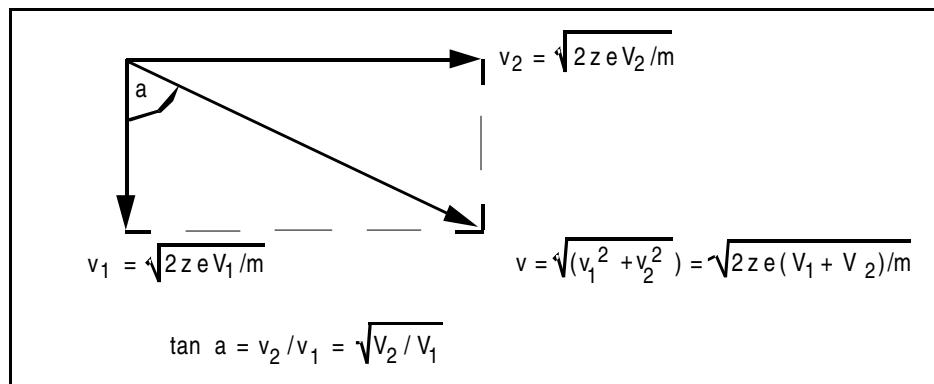


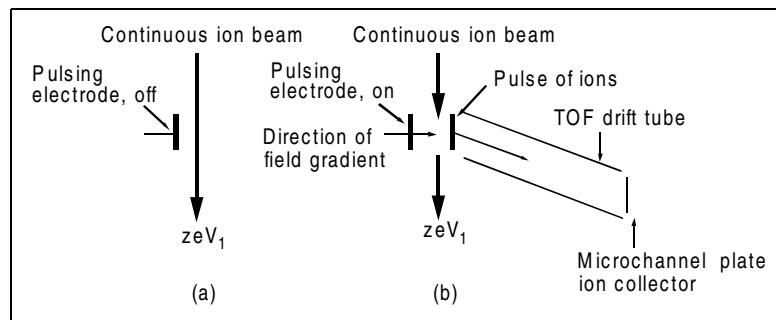
Figure 1 An ion beam is produced by accelerating ions of charge ze from an ion source through an accelerating voltage of V_1 volts so that they have kinetic energy corresponding to zeV_1 and a velocity $v_1 = \sqrt{(2zeV_1/m)}$. In an orthogonal acceleration chamber, the ions are subjected to a pulsed electric field with accelerating voltage of V_2 volts, which gives them additional kinetic energy zeV_2 with a velocity $v_2 = \sqrt{(2zeV_2/m)}$ at right angles to their original direction. The ion beam has a resultant energy of $ze(V_1+V_2)$, and a resultant velocity component $v = \sqrt{(2ze(V_2+V_1)/m)}$, in a direction α degrees from the initial direction, where $\tan \alpha = \sqrt{(V_2/V_1)}$.

The physical basis of orthogonal TOF

Consider a stream of ions emitted from an ion source as a beam. The ions are produced continuously and are first accelerated through an electric field of V_1 volts. If the number of charges on an ion is z and e is the charge on the electron then the energy acquired by an ion after acceleration through a field of V_1 volts is zeV_1 . This energy must be equal to the kinetic energy ($mv^2/2$) gained by the ion, where m is the mass of the ion and v is its final velocity. Thus, $v = \sqrt{(2zeV_1/m)}$ and its momentum, $mv = \sqrt{(2zemV_1)}$. Therefore, the beam consists of a range of ions having momenta proportional to the charge, mass and accelerating voltage. As the beam is produced continuously, there is no separation of ions in time (no temporal separation). This is the beam shown as an arrow with velocity $\sqrt{(2zeV_1/m)}$ in Figure 1. A magnetic sector, for example would separate the ions in space because the effect of the magnetic field is to bend the flight path in proportion to mass, charge and accelerating voltage (see Back-to-Basics, *Ion Optics*).

Now consider ions emitted from an ion source not as a beam but as a pulse, so that all ions are accelerated through the potential V_1 , applied as a pulse. Thus all ions start out from the ion source at exactly the same time and then pass along a flight tube of length d . The times taken for the ions to reach a collector is given by $t = d/v$, where $v = \sqrt{(2zeV_1/m)}$, as above. This is the basis of the time-of-flight instrument (TOF), in which ions of different m/z values are separated according to t .

Let there be an electrode placed so that its (pulsed) electric field gradient (direction) is at right angles to the continuous beam. If the electric potential at this point is V_2 volts, then a pulse of ions will be given additional energy zeV_2 , and a velocity $\sqrt{(2zeV_2/m)}$ in a direction at right angles to the main beam. The vectorial resultant velocity of the ions and direction of travel of the pulsed set are shown in Figure 1. It can be seen that, effectively, a section of the main ion beam is selected and pulsed away (Figure 2). Ions in this pulsed set all start at the same instant and can be timed by a TOF instrument. The flight times give m/z values and the numbers of ions give the abundances; the two are combined to give a mass spectrum.

**Figure 2**

In (a), the pulsing electrode is switched off and a continuous ion beam of energy, zeV_1 , passes by it.

In (b), the electrode has been pulsed for a few microseconds, with a field gradient at right angles to the main beam. This has caused a section of the ion beam to travel in the direction shown, the direction being determined by the magnitudes of the voltages V_1 and V_2 (see Figure 1). The detached segment of the main beam enters the flight tube of a TOF instrument. The m/z values are determined from the times taken for the ions to reach the microchannel plate ion collector after initiation of the pulse.

Pulsed main beams of ions

Although the above has considered only the use of a continuous main ion beam, which is then pulsed, it is not necessary for the initial beam to be continuous; it too can be pulsed. For example, laser desorption uses pulses of laser light to effect ionization and the main ion beam already consists of pulses of ions passing the orthogonal pulsing electrode. As shown below, this is of no consequence because these pulses of ions can again be directed into a TOF flight tube just as though they had formed part of a continuous ion beam.

Rate of application of the pulsed field gradient

Clearly, the pulsing electrode may be turned on and off at any frequency chosen but there are some constraints on the frequencies actually used. At the fastest, there is little point in pulsing the electrode at such a rate that one lot of ions has not had time to travel the length of the flight tube before the next lot is on its way. With the physical dimensions of typical flight tubes and the magnitudes of accelerating voltages commonly used in mass spectrometers, an upper limit of about 30 kilohertz is found. The slowest rate of pulsing the electrode is almost anything! At 30 KHz, the TOF instrument can measure one mass spectrum every 33 microseconds or, put another way, in one second the TOF instrument can accumulate and sum 30,000 spectra. Little wonder that the acquisition of a spectrum appears to be instantaneous on the human time scale.

Microchannel plate ion collector

A fuller description of the microchannel plate forms part of a separate Back-to-Basics guide, *Multipoint Collectors*. Briefly, ions travelling down the flight tube of a TOF instrument are separated in time. As each m/z collection of ions arrives at the collector, it may be spread over a small area of space (Figure 3). Therefore, so as not to lose ions, rather than have a single point ion collector, the collector is composed of an array of miniature electron multipliers (microchannels), which are all connected to one electrified plate so that, no matter where an ion of any one m/z value hits the front of the array, its arrival is recorded. The microchannel plate collector could be crudely compared to a satellite TV dish receiver in that radio waves of the same frequency but spread over an area are all collected and recorded at the same time; of course, the multichannel plate records the arrival of ions not radio waves.

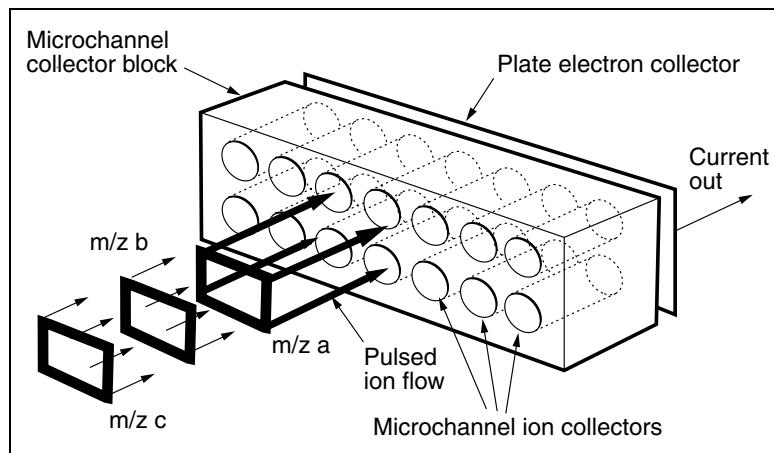


Figure 3 The diagram represents a flow of ions of m/z a , b , c , etc., travelling in bunches towards the front face of a microchannel array. After each ion strikes the inside of any one microchannel, a cascade of electrons is produced and moves towards the back end of the microchannel, where they are collected on a metal plate. This flow of electrons from the microchannel plate constitutes the current produced by the incoming ions (often called the ion current but actually a flow of electrons). The ions of m/z a , b , c , etc., are separated in time and reach the front of the microchannel collector array one set after another. The time at which the resulting electron current flows is proportional to $\sqrt{m/z}$ and the strength of the current represents the abundance of ions striking the microchannel plate collector.

Resolution by m/z value

Since the microchannel plate collector records the arrival times of all ions the resolution depends on the resolution of the TOF instrument and on the response time of the microchannel plate. A microchannel plate with a pore size of 10 µm or less has a very fast response time of less than 2 nanoseconds. The TOF instrument with microchannel plate detector is capable of unit mass resolution to beyond m/z 3000.

MS/MS operation

Figure 4 shows a diagrammatic representation of a typical MS/MS experiment, in which a main ion beam selected for ions (precursor ions) of mass m and having kinetic energy, zeV , has been directed into a collision cell so as to cause fragmentation into two new species (products) of mass m_1 and m_2 with charges z_1 and z_2 respectively (z_1 or z_2 may be zero). The kinetic energies of the product ions can be written as z_1eV' and z_2eV'' respectively. Without setting a pulsed electric field gradient orthogonal to the main beam, these fragment ions continue straight on. Application of a pulsed voltage to the electrode gives the ions a velocity component at right angles to their original direction. The vectorial resultant velocities form angles α_1 and α_2 to the original direction of the beam (see Figure 1). Although now directed along different paths (Figure 4), both beams of fragment ions strike the wide microchannel plate. The times of arrival at the plate are proportional to the $\sqrt{(m/z)}$ values of the masses involved and a mass spectrum of the product ions resulting from collisional activation is produced. It should be recalled that, after a single collision, the momenta and kinetic energies of product ions are different from the momenta or kinetic energies of the precursor ions but the velocities of the product ions are equal and equal to that of the initial precursor ions. There is no change in velocities of ions on fragmentation, only of momentum and kinetic energy. The TOF section can measure this mass spectrum in the normal fashion but, of course, it is a mass spectrum of the product ions resulting from fragmentation of precursor ions in the collision cell.

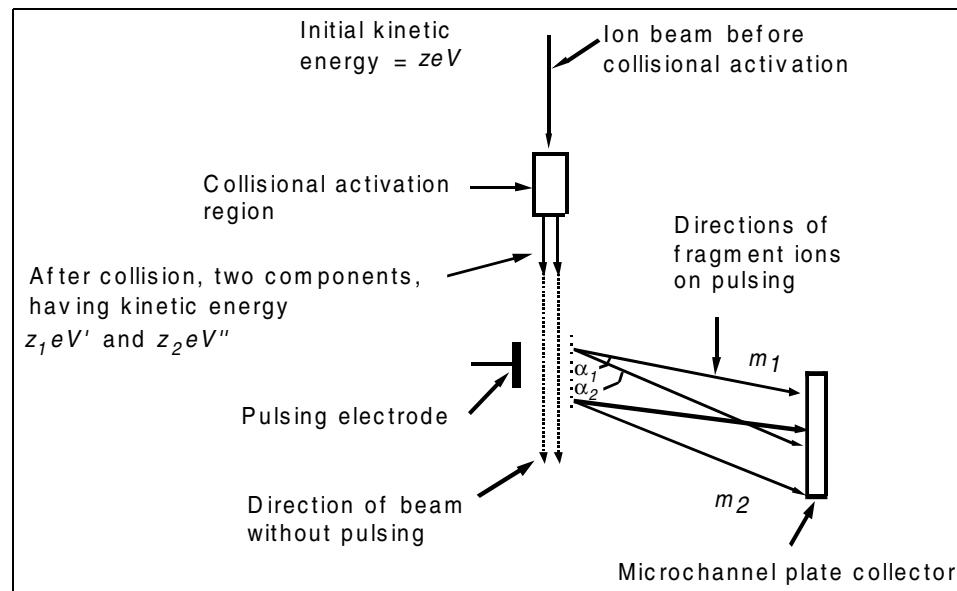


Figure 4 The diagram shows a mass-selected main ion beam (precursor ions) of kinetic energy, zeV , entering a collisional activation region and being fragmented to produce two fragment (product) ions, having kinetic energies equal to z_1eV' and z_2eV'' . If no electric field is pulsed onto the electrode, the ions continue straight on. If a pulsed electric field is applied, ions of energy z_1eV' will be deflected through an angle α_1 and the ions of energy z_2eV'' will be deflected through an angle α_2 and into a TOF analyser tube. Both deflected beams are detected at the microchannel plate collector.

Advantages of orthogonal TOF arrangements

As indicated above, specific orthogonal TOF instruments are covered in greater detail in the section on hybrid instruments. However, it may be noted that the orthogonal TOF instrument provides significant advantages for MS/MS operation in the examination of trace quantities of materials and as an adjunct to instruments in which the ion sources do not yield a steady ion current but rather pulsed sets of ions (laser desorption, radioactive desorption, sputtering). Even for ‘continuous’ ion sources, vagaries of the ion current are smoothed out through the accumulation of, say, 30,000 spectra at 33 microsecond time intervals in a space of one second. The summed spectra are printed out as one mass spectrum. There is often a significant gain in signal-to-noise ratio for the orthogonal TOF system.

Conclusion

‘Snapshots’ of a beam of ions may be taken by accelerating in pulses, sections of the beam, away from the main stream. The accelerating voltage to do this is applied as electric field pulses on an electrode. The pulsed field gradient is at right angles (orthogonal) to the direction of the main beam. The pulsed ions are analysed in a time-of-flight tube and collected by a microchannel plate detector. The orthogonal TOF arrangement may be used in connection with a variety of other kinds of mass spectrometer to produce useful hybrid instruments. There are distinct advantages to these hybrids, compared with the separate instruments alone.

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Back to Basics Section E: Ion Collectors

CHAPTER E1

POINT ION COLLECTORS (DETECTORS)

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Quick Guide

- A mass spectrum is a plot of mass-to-mass-charge (m/z) values for ions versus their abundances.
- A mass spectrometer analyser disperses ions according to their various m/z values.
- Recording of the dispersed ion beams can take place simultaneously across a plane, as in an array detector or, as described here, by being brought to a focus at one point sequentially.
- By placing a suitable detector at the focus (a point detector), the arrival of ions can be recorded.
- Point detectors are usually a Faraday cup (a relatively insensitive device) or, more likely an electron multiplier (a very sensitive device) or, less likely, a scintillator (another sensitive device).
- Arrival of ions, which necessarily have a positive or negative charge, causes an electric current to flow either directly, as in the Faraday cup, or indirectly as in the electron multiplier and scintillator detectors.
- The flow of electric current marks the arrival of ions and its magnitude marks the abundance of ions arriving at any m/z value.
- The electric current is used to drive a recorder of some kind which may be an oscilloscope or pen recorder or galvanometer UV recorder.
- More likely nowadays, the analogue electrical signal will be digitized and processed by a computer (data) system.
- A data system stores the mass spectrum until required when it can be printed out (at leisure) or viewed (immediately) on the computer screen.

Summary

Having been separated according to their m/z values, ions can be focused sequentially at a point where there is a detector, usually a Faraday cup or electron multiplier, which generates an electrical current proportional to the numbers of ions arriving.

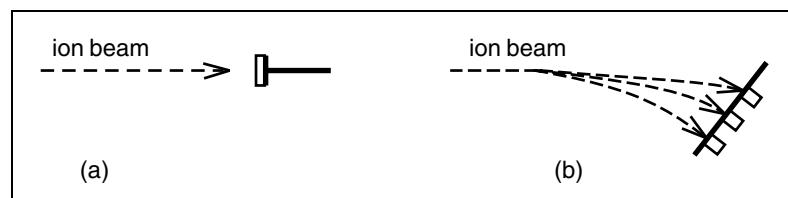


Figure I (a) Point ion collector. The ion beam is focused at a point, as shown, and ions of different m/z value are directed onto the collector sequentially in the time domain.
(b) Array ion collector. The ion beam is dispersed and all m/z values are recorded simultaneously in a space domain.

POINT ION COLLECTORS

Introduction

Ion detectors can be separated into two classes - those which detect the arrival of all ions sequentially at one point (a point ion detector) and those which detect the arrival of all ions simultaneously along a plane (an array detector). In this guide only point detectors are discussed. The Back-To-Basics guide on array collectors (detectors) should be consulted for the other kind.

All mass spectrometers analyse ions for their mass-to-charge ratios (m/z values) by separating the individual m/z and then recording the numbers (abundance) of ions at each m/z value to give a mass spectrum. Quadrupoles allow ions of different m/z values to pass sequentially, viz., ions at m/z 100, 101, 102 for example will pass one after the other through the quadrupole assembly so that first m/z 100 is passed, then 101, then 102 (or vice versa). Therefore, the ion collector (or detector) at the end of the quadrupole assembly needs only to cover one point or focus for a whole spectrum to be scanned over a period of time (Figure 1a). This type of point detector records ion arrivals in a time domain, not a spatial one.

A magnetic sector instrument separates ions according to their m/z values but, unlike the quadrupole, by dispersing them in space (Figure 1b). Having dispersed the ions, their arrival can be recorded over a region of space (array detection) or, by increasing (or decreasing) the magnetic field, the ions can be brought sequentially to a focus (point detection). Either point or array detectors are used with magnetic instruments.

Other types of mass spectrometer may use point, array or both types of collector. The time-of-flight instrument uses a point ion collector; an ion trap may record ion arrivals either sequentially in time or all at once; a Fourier transform ion cyclotron resonance (FT-ICR) instrument can record ion arrivals in either time or frequency domains which are interconvertible (by the Fourier transform technique).

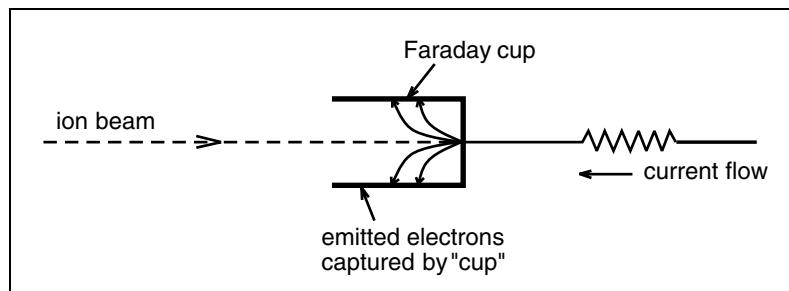


Figure 2 Ions travelling at high speed strike the inside of the metal (Faraday) cup and cause secondary electrons to be ejected. This production of electrons constitutes a temporary flow of electric current until the electrons have been recaptured.

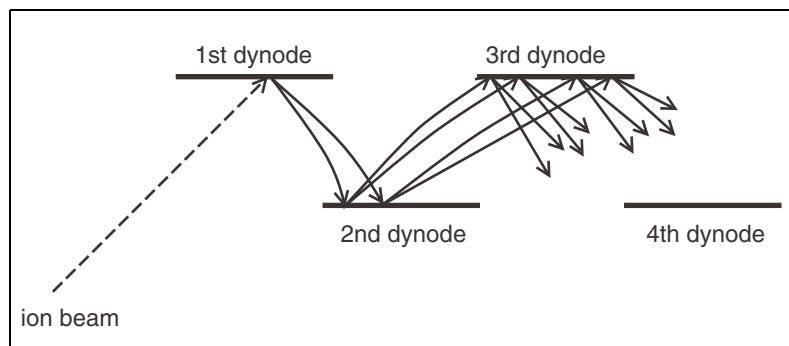


Figure 3 An incident ion beam is shown here to cause two electrons to be emitted from the first dynode. These electrons are accelerated to the second dynode where each causes two more electrons (four in all) to be ejected. These in turn are accelerated to a third dynode and so on, eventually reaching, say, a tenth dynode by which time the initial two electrons have become a shower of 2^9 electrons.

Types of Point Ion Collector

Three main types are in use for quadrupole, magnetic sector and time-of-flight instruments and are discussed here. FT-ICR requires separate study and will not be covered.

(a) Faraday Cup Ions carry positive or negative electric charges and, on arrival at an earthed metal plate they are neutralized either by accepting or donating electrons. The resulting flow of electrons constitutes a tiny electric current which can be amplified and used to drive a recording device. Such a simple plate collector is relatively inefficient because the ions are also often travelling at high velocity. Fast moving ions on striking a metal surface cause a 'shower' of electrons to be emitted (secondary electrons). Therefore, by using a 'cup' collector rather than a simple 'plate', the secondary electrons can be collected (Figure 2). Thus, one positive ion arriving at a (Faraday) cup collector needs one electron for neutralisation but causes several electrons to be emitted; this provides a 'gearing' or amplification - several electrons for each ion. The Faraday cup detector is simple and robust and is used in situations in which high sensitivity is not required.

(b) Electron Multiplier As mentioned above, a particle such as an ion travelling at high speed when it strikes a metal surface causes a number of secondary electrons to be ejected. This principle is made use of in the electron multiplier (Figure 3). Ions are directed onto the first plate (dynode) of an electron multiplier. The ejected electrons are accelerated through an electric potential so that they strike a second dynode. Suppose each ion collision causes ten electrons to be ejected and, at the second dynode, each of these electrons causes ten more to be ejected towards a third dynode. In such a situation, arrival of just one ion causes $10 \times 10 = 10^2 = 100$ electrons to be ejected from the second dynode, an amplification of 100. Commercial electron multipliers routinely have 10, 11 or 12 dynodes so that amplifications of 10^6 are readily available, the final flow of electrons providing an electric current which can be further increased by ordinary electronic amplification. These multipliers provide highly sensitive ion detection systems. They work under a vacuum and can be connected directly to the mass spectrometer. Only electrical potentials on the dynodes are needed to make them work and so they are very robust.
Eventually, multipliers become less sensitive and even fail because of surface contamination caused by the imperfect vacuum in the mass spectrometer and the impact of ions on the surfaces of the dynodes.

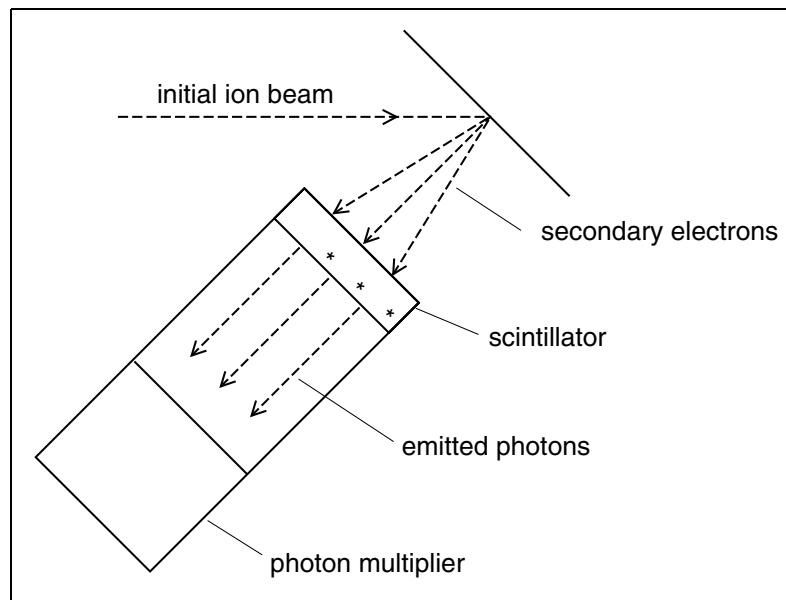


Figure 4 An incident ion beam causes secondary electrons to be emitted and these are accelerated onto a scintillator (compare with the operation of a TV screen). The photons which are emitted (like the light from a TV screen) are detected not by eye but with a highly sensitive photon detector (photon multiplier) which converts the photon energy into an electric current.

(c) Scintillator This ion collector is sometimes known as the 'Daly' detector and is useful for studies on metastable ions. The principle of operation is illustrated in Figure 4. As with the first dynode of an electron multiplier, the arrival of a fast ion causes electrons to be emitted and these are accelerated towards a second 'dynode'. In this case, the dynode consists of a substance (a scintillator) which emits photons (light). The emitted light is detected by a commercial photon multiplier placed behind the scintillator screen and is converted finally into an electric current. Since photon multipliers are very sensitive, high gain amplification of the arrival of a single ion is achieved. These detectors are also important in studies on metastable ions.

Conclusion

An ion beam causes secondary electrons to be ejected from a metal surface. These secondaries may be measured as an electric current directly through a Faraday cup or, indirectly after amplification, as with an electron multiplier or a scintillation device. These devices are located at a fixed 'point' in a mass spectrometer and all ions are focused on that point - hence the name, point ion collector. In all cases, the resultant flow of an electric current is used to drive some form of recorder or is passed to an information storage device (data system).

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Back to Basics Section E: Ion Collectors

CHAPTER E2

ARRAY COLLECTORS (DETECTORS)

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Quick Guide

- A mass spectrum is a plot of mass-to-charge (m/z) values for ions versus their abundances.
- A mass spectrometer analyser disperses ions according to their various m/z values either in space or in time.
- Recording of a dispersed ion beam can take place either at a point (see Point Ion Collectors) or across a plane, as in the array collector described here.
- An array collector is a collection of point collectors (elements), assembled in a plane.
- Ions of any particular m/z value are collected at one of the small point ion detectors; ions of larger or smaller m/z values are collected at other point collectors placed on either side.
- By having a large number of point ion collectors in a line in a plane, recording of many different m/z values can be made at the same time (concurrently rather than sequentially as with a single point ion collector).
- According to the dispersion achieved at the array plane, m/z values may be separated by fractions of a unit mass, by unit mass or only by tens of mass units.
- Each element of an array detector is essentially a small electron multiplier as with the point ion collector but much smaller and often shaped somewhat like a snail shell.
- The arrival of ions at the opening of one of the array elements causes a shower of electrons to pass to the end of the collector where they are recorded as a current flow, which is usually amplified.
- The magnitude of the current flow is proportional to the number of ions arriving at the array element per unit time.
- The amplified signal may be recorded directly by analogue means but, more likely, the signal is digitized and processed by a computer (data system).

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- The resolution achievable by an array assembly depends critically on the number of elements in the array, the separation of one element from another and the degree of dispersion of the ions in the ion beam.
- By collecting all ions at the same time, a mass spectrum can be obtained instantaneously instead of over a period of time as with a point ion collector.
- Array detectors are particularly useful for detecting ions from either a very small amount of a substance or when ionization is not continuous but intermittent.

Summary Having dispersed an ion beam in space according to the various m/z values of the ions, the ions are collected simultaneously by a planar assembly of small electron multipliers. All ions over a specified mass range are detected at the same time, giving the array detector an advantage for analysis of very small quantities of any one substance or where ions are produced intermittently during short time intervals.

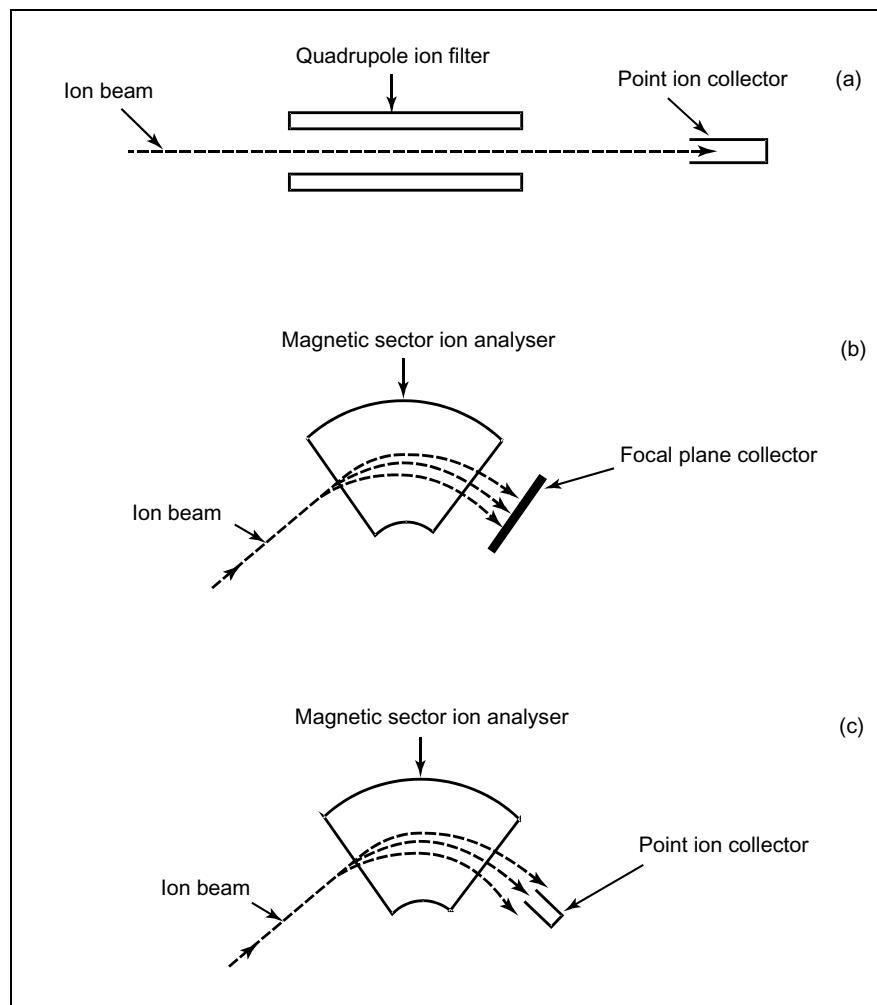


Figure I In (a), ions of different m/z values traverse a quadrupole mass filter one after another and only a point ion collector is needed. In (b), the ion beam is shown dispersed in space, each m/z value being brought to a focus at a point that lies in a focal plane. A photographic plate or an array detector lying along the focal plane can detect all ions at the same time. In (c), the same dispersed ion beam as in (b) is brought to a focus at one point by changing the strength of the magnetic field and, in this case, a point ion collector would be used.

ARRAY COLLECTORS (DETECTORS)

Introduction

Ion detectors may be separated into two classes – those that detect the arrival of all ions sequentially at a point (a point ion collector) and those that detect the arrival of all ions simultaneously (array collector). In this guide, only array collection (detection) is discussed. The Back-to-Basics guide on point ion collectors should be consulted for the other kind.

All mass spectrometers analyse ions for their mass-to-charge ratios (m/z values) and simultaneously for the abundances of ions at any one m/z value. By separating the ions according to m/z and measuring the ion abundances, a mass spectrum is obtained. Quadrupole mass spectrometers (mass filters) allow ions at each m/z value to pass through sequentially, e.g., ions at m/z 100, 101, 102 will pass one after the other through the quadrupole assembly so that first m/z 100 is transmitted, then m/z 101, then m/z 102 and so on. Therefore, the ion collector at the end of the quadrupole unit needs only to cover one point or focus in space (Figure 1a) and a complete mass spectrum is recorded over a period of time; the ions arrive at the collector sequentially and ions are detected in a time domain, not in a spatial domain.

A magnetic sector instrument separates ions according to their m/z values but, unlike the quadrupole, by dispersing them in space (Figure 1b). Having dispersed the ions, their arrival can be recorded simultaneously in space (array or photographic plate focal plane detection, Figure 1b) or, by increasing or decreasing the strength of the magnetic field, the ions can be brought sequentially to a focus at a point ion collector (Figure 1c). It is array collection which is discussed here.

Other types of mass spectrometer may use point or array or both types of ion detection. A time-of-flight mass spectrometer collects ions sequentially and uses a point detector. A magnetic sector instrument may collect ions sequentially (point detector) or simultaneously (array detector).

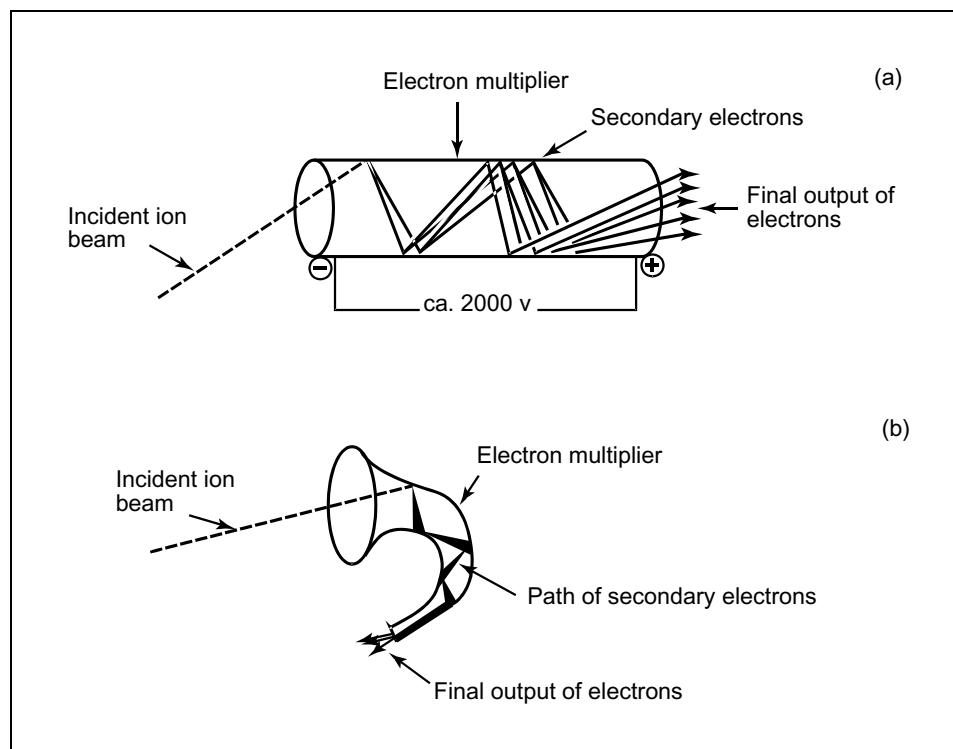


Figure 2 Diagram (a) represents a straight electron multiplier which has a semiconducting inner surface. There is an electrical potential of about 1000 to 2000 volts between the ends of the tube, which is shown set up for detection of positive ions. Incident ions striking the inner surface cause secondary electrons to be emitted and these are accelerated by the high potential between the ends of the multiplier tube. In turn, the electrons strike the inner wall and each causes more electrons to be emitted. Thus, as the electrons travel along the tube, striking the inner walls at intervals, more and more electrons are produced so that, for one incident ion, there will be a shower of electrons leaving the end of the multiplier.

Diagram (b) shows a different shape of electron multiplier tube, designed to stop back scatter of the secondary electrons which can cause excessive electrical noise. Otherwise, this design works on the same principle as the straight multiplier. This type of multiplier with no separate dynodes can be made in a wide variety of shapes and sizes and, importantly for an array collector, can be made small.

The original method for simultaneously recording a range of ions was to use a photographic plate which was placed in the focal plane (figure 1b) such that all ions struck the photographic plate simultaneously at different positions along the plate. This method of detection is now rarely used because of the inconvenience of having to develop a photographic plate. Essentially, the array collector has taken its place. Ion trap mass spectrometers may detect ions sequentially or simultaneously and, in some cases, may not use a formal ion collector at all; the ions can be detected by their different electric field frequencies in flight, according to m/z value.

Array detection

The array detector (collector) consists of a number of ion collection elements, arranged in a line; each element of the array is an electron multiplier.

An element of the array

The Back-to-Basics guide to electron multipliers should be consulted for greater detail on how they work. Where space is not a problem, a linear electron multiplier having separate dynodes to collect and amplify the electron current created each time an ion enters its open end can be used. For array detection, the individual electron multipliers must be very small so that they can be packed side-by-side into as small a space as possible. For this reason, the design of an element of an array is significantly different from that of a standard electron multiplier used for point ion collection, even though its method of working is similar. Figure 2a shows an electron multiplier (also known as a channeltron®) that works without using separate dynodes. It may be used to replace a dynode type multiplier for point ion collection but, because the channeltron can be made very small, it is suited to array ion collection where as many multipliers as possible, placed side by side, must be fitted into a relatively small space. The channeltron can be made in a variety of shapes, one of which is shown in Figure 2b.

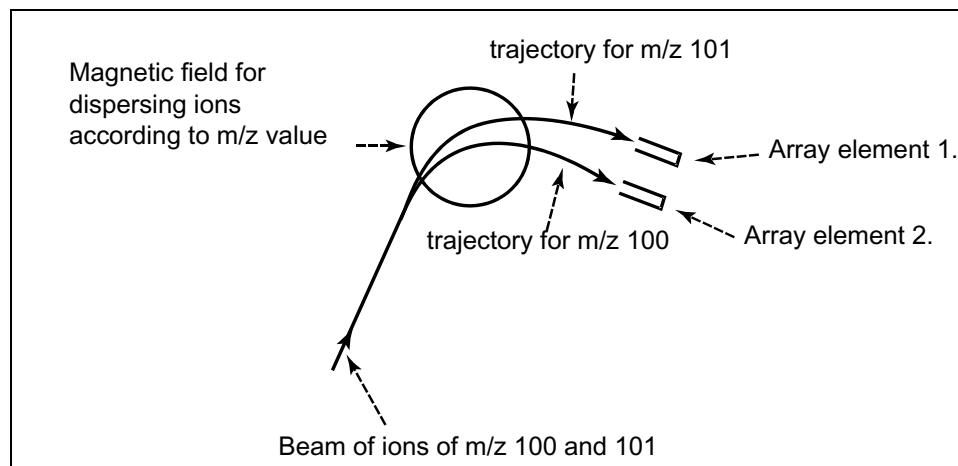


Figure 3 An ion beam containing just two types of ion of m/z values 100 and 101 dispersed in space on passing through a magnetic field. After dispersal, ions of individual m/z value 100 or 101 are focused at points close to the entries of two elements of an array collector. Each element of the array is a point ion collector.

For operation of a channeltron, a large electrical potential is maintained between the open and closed ends of the element. An ion travelling at high speed enters the mouth of the channeltron and strikes the internal wall, causing a shower of electrons to be given off; these electrons are accelerated through the electric potential but travel more or less in straight lines so that they soon strike the internal wall again (Figure 2a, b).

Each electron in the shower itself causes a second shower to be emitted. In this way, the electrons first produced travel down the channeltron rebounding from the walls and increasing in number. Eventually, for the arrival of one ion, hundreds or thousands of electrons arrive at the closed end of the array element where they constitute an electric current that can be measured and amplified. Often, this sort of electron multiplier is made into a curved shape (for example, that illustrated in Figure 2b) so as to reduce back-scattering of ions or electrons that would cause excessive electrical noise. Various operating modes can be used, such as analogue, digital, light coupled and so on but there is neither the space nor the need to discuss these here.

Separation of
array elements
(ion mass range)

Consider just two array elements as illustrated in Figure 3 and suppose an ion beam has been dispersed to give ions of m/z values 100 and 101. If the dispersion is correct for the array size, the ion of m/z 100 will enter the first element and the ion of m/z value will enter the second. Thus, at this level of dispersion, unit m/z values can be separated. Simple extrapolation to say 5 ions of different m/z values (Figure 4) and 10 array elements in a line shows that all of the ion m/z values and abundances can be measured simultaneously to give an instantaneous spectrum with integer m/z values. Further extrapolation indicates that more m/z values can be measured if there are more array elements. However, these do not come without cost and fitting a very large number of elements into a compact array becomes increasingly difficult. Therefore, a limited number of array elements is used, say 100, which means that sections of a mass spectrum can be measured instantaneously but, if it is required to measure the whole spectrum then it must be measured in sections at a time. Frequently, not all of a mass spectrum is needed and it is unnecessary to measure more than one region of the spectrum. Maybe only the molecular ion region needs to be covered.

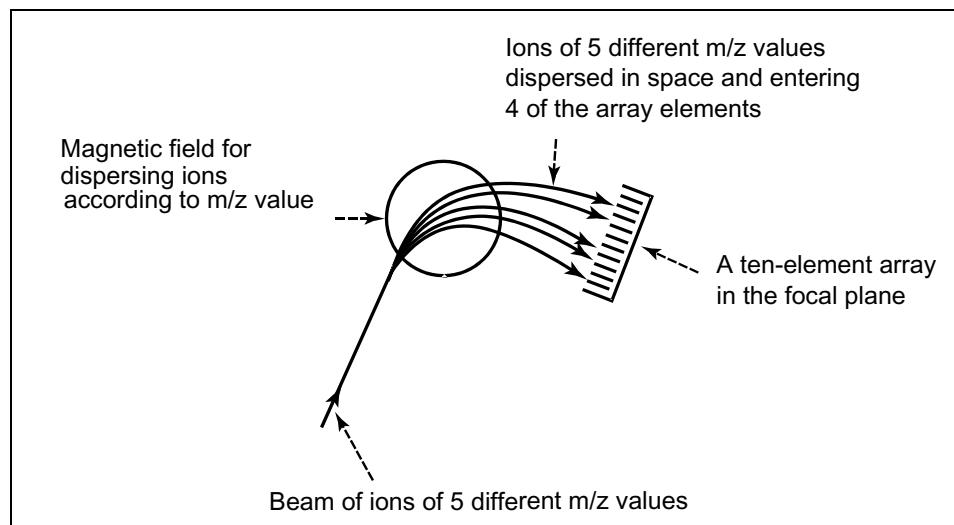


Figure 4 Ions of five different m/z values shown entering five elements of a ten-element array. All five ions would be recorded at the same time, with electrical outputs from the elements into which ions had entered and no output from those elements into which no ions had entered.

The array may not consist simply of one line of elements; there may be several such lines, one above the other. The single line is discussed here for the sake of simplicity.

Now, consider ions of m/z 100.1 and 100.2. If these are dispersed so as to enter the two elements of Figure 3 then the resolution improves to 0.1 mass unit but the *number* of m/z values that can be measured at the same time falls. In the 10 element array of Figure 4, for 0.1 mass resolution, only the mass range of m/z 100 to 101 would be covered, instead of the 100 to 110 for unit mass resolution.

As the number of elements in the array does not change, this means that the less of the spectrum that can be covered, the higher the resolution. Conversely, the lower the resolution, the greater the mass range that may be scanned. Thus, arrays can be used to cover wide regions of a mass spectrum at low resolution but only very small regions at high resolution.

Dynamic range (ion abundance)

An electron multiplier used with point ion detection should ideally have a wide dynamic range of response for numbers of ions arriving for collection, *viz.*, it should be almost equally efficient at detecting a few ions as detecting tens of thousands of ions (dynamic range) with a fair linear response between numbers of ions arriving and the final output from the multiplier. This would mean that the sizes of peaks observed in a mass spectrum, no matter whether small or big, would relate reasonably well to the actual number of ions they are meant to represent (100 ions should be represented by a peak 100 times bigger than that for 1 ion). The elements of an array detector are small and more easily saturated by the arrival of large numbers of ions than is the much larger single electron multiplier. This means that the dynamic range of the array is generally worse than that of a single point ion collector. For most practical situations this difference is not too important but should be borne in mind. It might also be worth noting here that, as the mass of an ion increases, the electrical response of any electron multiplier falls and, at high mass (say hundreds of mass units), the inherent sensitivity is very much less than for low mass. This effect is due to the fact that ions of large m/z values are usually travelling much slower than ions of small m/z values and therefore produce fewer electrons on initial impact with the multiplier.

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Uses of array collectors

The major advantage of array detectors over point ion detectors lies in their ability to measure a *range* of m/z values and the corresponding ion abundances *all at one time*, rather than sequentially. For example, suppose it takes 10 milliseconds to measure one m/z value and the associated number of ions (abundance). To measure 100 such ions sequentially with a point ion detector would necessitate 1000 milliseconds (1 second); for the array detector, the time is still 10 milliseconds because all ions arrive at the same time. Therefore, when it is important to be able to measure a range of ion m/z values in a short space of time, the array detector is advantageous.

There are two common occasions when rapid measurement is preferable. First, with ionization sources such as those using laser desorption or radionuclides. A pulse of ions is produced in a very short interval of time, often of the order of a few nanoseconds. If the mass spectrometer takes 1 second to attempt to scan the range of ions produced then, clearly, there will be no ions left by the time the scan has completed more than a few nanoseconds (ion traps excluded). If a point ion detector were to be used for this type of pulsed ionization then, after the beginning of the scan, no more ions would reach the collector because there would not be any left! The array collector overcomes this difficulty by detecting the ions produced all at the same instant.

A second use of arrays arises in the detection of traces of material introduced into a mass spectrometer. For such very small quantities, it may well be that, by the time a scan has been carried out by a mass spectrometer having a point ion collector, the tiny amount of substance may have disappeared before the scan has been completed. An array collector overcomes this problem. Often, the problem of detecting trace amounts of a substance using a point ion collector is overcome by measuring not the whole mass spectrum but only one 'characteristic' m/z value (single ion monitoring or single ion detection). However, unlike array detection, this single ion detection method does not provide the whole spectrum and an identification based on only one m/z value may well be open to misinterpretation and error.

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Conclusion An array ion collector (detector) consists of a large number of miniature electron multiplier elements arranged side by side along a plane. Point ion collectors gather and detect ions sequentially (all ions are focused at one point *one after another*) but array collectors gather and detect all ions simultaneously (all ions are focused onto the array elements *at the same time*). Array detectors are particularly useful for situations in which ionization occurs in a very short space of time, as with some ionization sources or with trace quantities of any substance. For these very short time-scales, only the array collector can measure a whole spectrum or part of a spectrum satisfactorily in the time available.

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Back to Basics Section E: Ion Collectors

CHAPTER E3

COMPARISON OF MULTIPOINT ION COLLECTORS

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Quick Guide

- A mass spectrum is a plot of mass-to-charge (m/z) values for ions versus their abundances.
- A mass spectrometer analyser disperses ions either in space or in time according to their various m/z values.
- Recording of a dispersed ion beam can take place either at a point (see Point Ion Collectors in this series) or across a plane, as in the multipoint collectors discussed here.
- A multipoint collector is an assembly (array) of single point collectors (elements), packed closely together in a plane.
- Ions arrive at one end of each element of a multipoint collector and trigger a growing cascade of electrons, which moves towards the opposite end and is detected electronically. The resulting electric current corresponds to the ion current frequently referred to in publications on mass spectrometry.
- The strength of the ion current relates to the number of ions per second arriving at the collector plate and a mass spectrum can be regarded as a 'snap-shot' of the current taken over a definite period of time. Because of the finite time taken to produce a mass spectrum, it is a record of the abundances of ions (often mistakenly called intensities of ions).
- Thus, a mass spectrum records ion abundances in one dimension. In the second dimension, it records m/z ratios. The mass spectrum is a record of m/z values of ions and their abundances.
- In a mass spectrometer, ions may arrive at a multipoint collector as a spatially dispersed beam. This means that all ions of different m/z values arrive simultaneously but separated in space according to each m/z value. Each element of the array, depending on its position in space, detects one particular m/z value (see Array Collectors in this series).
- Alternatively in a mass spectrometer, ions may arrive at a multipoint collector as a temporally dispersed beam. This means that, at any point in time, all ions of the same m/z value arrive simultaneously and different m/z values arrive at other times. All elements of this collector detect the arrival of ions of one m/z value at any one instant of time. This type of detector, which is also an array, is called a microchannel plate collector of ions.

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- To differentiate their functions and modes of operation, the array collector of spatially dispersed m/z values is still called an array collector for historical reasons, but the other multipoint array detector of a temporally dispersed range of m/z values is called a microchannel plate (typically used in time-of-flight instruments).
- Each element of an array or a microchannel plate ion collector is essentially one electron multiplier, similar in operation to the type used for a point ion collector but very much smaller.
- Unlike the array collector, with a microchannel plate all ions of only one m/z value are detected simultaneously and instrument resolution does not depend on the number of elements in the microchannel array or on the separation of one element from another. For a microchannel plate, resolution of m/z values in an ion beam depends on their being separated in time by the analyser so that their times of arrival at the plate differ.
- In a beam of ions separated in time according to m/z value, the total time taken for ions of different m/z values to arrive at a microchannel plate is so short (about 30 microseconds) that the spectrum appears to have been obtained instantaneously. Thus, for practical purposes, the array and microchannel plate collectors produce an instantaneous mass spectrum, even though the first detects a spatially dispersed set of m/z values and the second detects a temporally dispersed set.
- Both types of multipoint array are particularly useful for detecting ions produced either from a very small amount of a substance or when ionization is not continuous but intermittent.

Summary

After the analyser of a mass spectrometer has dispersed a beam of ions in space or in time according to their various m/z values, they may be collected by a planar assembly of small electron multipliers, called an array in one case (spatial separation) and a microchannel plate in the other (temporal separation). With both multipoint assemblies all ions over a specified mass range are detected at the same time or apparently at the same time, giving these assemblies distinct advantages over the single point collector in the analysis of very small quantities of a substance or where ions are produced intermittently during short time intervals.

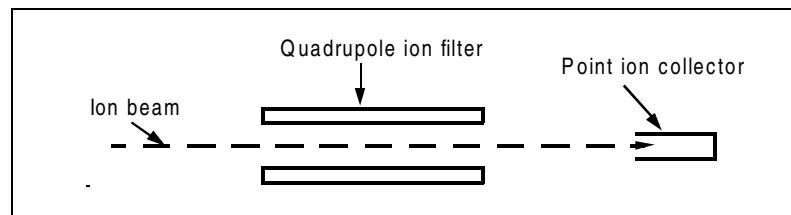


Figure I Ions of different m/z values pass sequentially in time through the quadrupole mass filter to reach an in-line, single point ion collector.

COMPARISON OF MULTIPOINT COLLECTORS (DETECTORS) OF IONS: ARRAYS AND MICROCHANNEL PLATES

Introduction All mass spectrometers analyse ions for their mass-to-charge ratios (m/z values) and simultaneously for the abundances of ions at any one m/z value. Once separated by m/z value, the ions must be detected (collected) and their numbers (abundances) measured for each m/z value. The resulting chart of m/z value versus abundance constitutes a mass spectrum.

In modern mass spectrometry, ion collectors (detectors) are generally based on the electron multiplier and may be separated into two classes - those collectors that detect the arrival of all ions sequentially at a point (a point ion collector) and those that detect the arrival of all ions simultaneously (an array or multipoint collector). In this guide, a comparison is made of the uses of single and multipoint ion collectors. The Back-to-Basics guides on Point Ion Collectors and Array Collectors should be consulted for more detailed discussions of their construction and operation. In some forms of mass spectrometry other methods of ion detection may be used, as with ion cyclotron instruments but these are not considered here.

Quadrupole mass spectrometers (mass filters) allow ions at each m/z value to pass through the analyser sequentially, viz., ions at m/z 100, 101, 102 for example are allowed to pass one after the other through the quadrupole assembly so that first m/z 100 is transmitted, then m/z 101, then m/z 102 and so on. Therefore, the ion collector at the end of the quadrupole unit needs only to cover one point or focus in space and can be placed immediately behind the analyser (Figure 1). A complete mass spectrum is recorded over a period of time (temporally), which is set by the voltages on the quadrupole analyser. In this mode of operation, the ions are sent to the collector one m/z value after another; the m/z values are said to be scanned sequentially. The resolution of m/z values is dependent solely on the analyser and not on the detector. The single point collector is discussed in detail in this series under the title, Point Ion Collectors (Detectors).

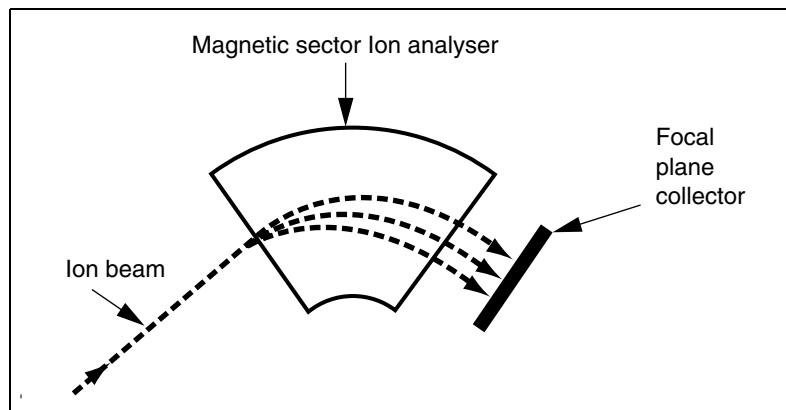


Figure 2 Ions of differing m/z values are dispersed by a magnetic sector and reach foci, which are distributed along a focal plane.

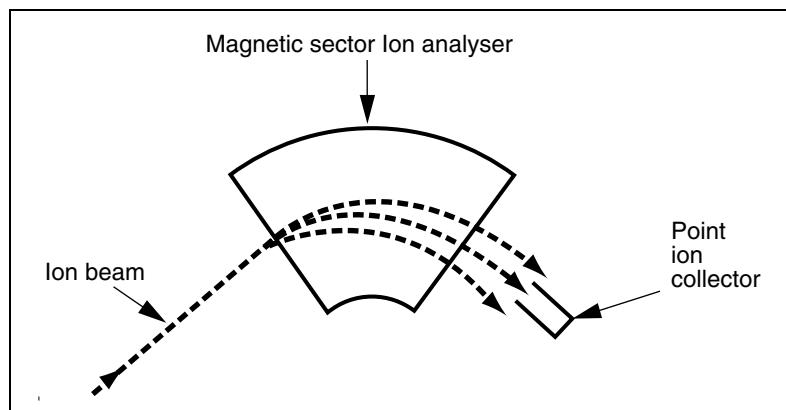


Figure 3 By adjusting the magnetic field, the dispersed ion beam in Figure 2 can be moved up or down so that ions of specific m/z values can be focused at a point ion collector.

Magnetic sector instruments separate ions according to their m/z values by dispersing them in space (Figure 2). By changing the strength of the magnetic field, the ions can be focused one after another according to m/z values at one point ion in space and collected over a period of time (Figure 3); this mode of operation needs only a single point detector, as for the quadrupole instrument. For this last collecting of ions in a time domain, resolution depends only on the analyser. Alternatively, having dispersed the ions in space (resolved them according to m/z value), all can be detected at the same time over a section of space by using an array of single point detectors (the focal plane collector in Figure 2). The array system is discussed in this series of Back-to-Basics under the title, *Array Collectors (Detectors)*. With array detection, resolution of m/z values depends both on the analyser and the collector. Historically, the method for recording ions dispersed in space was to use a photographic plate, which was placed in the focal plane, such that all ions struck the photographic plate simultaneously but at different positions along the plate depending on m/z value. This method of detection is now rarely used because of the inconvenience of having to develop a photographic plate.

Other types of mass spectrometer may use point or array or both types of ion detection. Ion trap mass spectrometers may detect ions sequentially or simultaneously and, in some cases, may not use a formal electron multiplier type of ion collector at all; the ions can be detected by their different electric field frequencies in flight, according to m/z value.

There is another form of array called a microchannel plate detector. A time-of-flight mass spectrometer collects ions sequentially in time and may use a point detector but, increasingly, the time-of-flight (TOF) instrument uses a microchannel plate, most particularly in an orthogonal TOF mode. Because the arrays and microchannel plates are both essentially arrays or assemblies of small electron multipliers, there may be confusion over their roles. This present guide illustrates the differences in the two arrays.

Arrays and Microchannel Plates

In both forms, the collector consists of a number of single point ion detection elements, each of which is a very small electron multiplier.

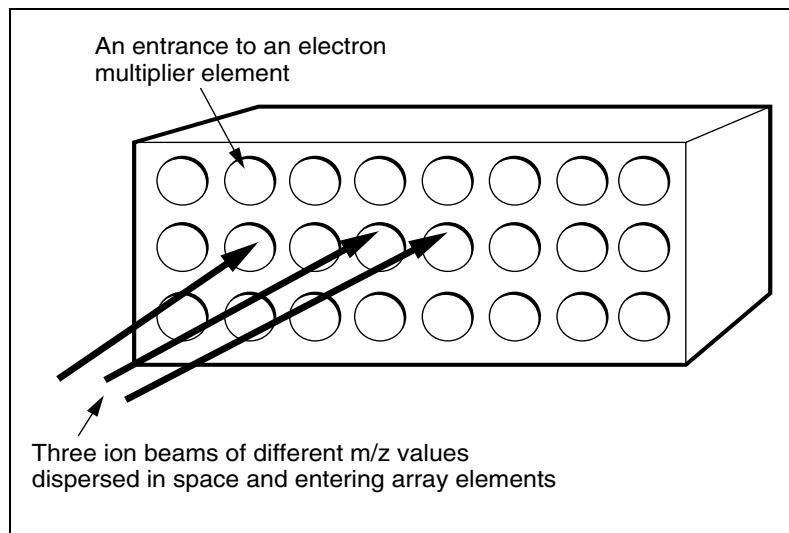


Figure 4 Idealized face view of a set of small electron multipliers arranged over a plane. Some typical individual multipliers are shown in later figures.

Each element is much smaller than the normal single electron multiplier and lots of them can be arranged close together as a planar array so as to cover a large area of space (Figure 4). The actual construction of the arrays is different as will be described below. The front face of the array contains the entrances or openings for each element, into which ions are deposited or collected. A fast moving ion striking the entrance to an element starts a cascade of electrons, which increases in size as the electrons ‘bounce’ off the walls of the element during their passage to its back end. At the back end of each element, it is either closed or open. In an array of closed elements, the end of each can be monitored for the cascading electrons, signalling the arrival of an ion or ions. In an array with open-ended elements, a cascade of electrons from any element is collected onto the same backing plate. Ions arriving anywhere in space over the face of the array are detected, viz., all of the elements are monitored as one. There is potential confusion in the use of the word ‘array’ in mass spectrometry.

Historically, ‘array’ has been used to describe an assemblage of small single point ion detectors (elements), each of which acts as a separate ion current generator. Thus, arrival of ions in one of the array elements generates an ion current specifically from that element. An ion of any given m/z value is collected by one of the elements of the array. An ion of different m/z value is collected by another element. Ions of different m/z value are dispersed in space over the face of the array and the ions are detected by m/z value at different elements (Figure 4).

An assemblage (array) of single point electron multipliers in a microchannel plate is designed to detect all ions of any single m/z value as they arrive separated in time. Thus, it is not necessary for each element of the array to be monitored individually for the arrival of ions. Instead, all of the back ends of the detection elements are connected together electronically. Thus, if ions of say m/z 100 arrive at some time, t , but are spread spatially over the face of the array, they are all detected simultaneously, even though some may be collected by one element, others by another element, and so on. There is no discrimination in m/z value. That separation of ions by m/z must be effected in time by the analyser, usually a TOF instrument (Figure 5).

To avoid confusion, the word ‘array’ is now used to describe an assemblage of small single point detectors, which remain as individual ion monitoring elements and ‘microchannel plate’ is used to describe an assemblage of small single point detectors, all of which are connected so as to act as a single large monitoring element.

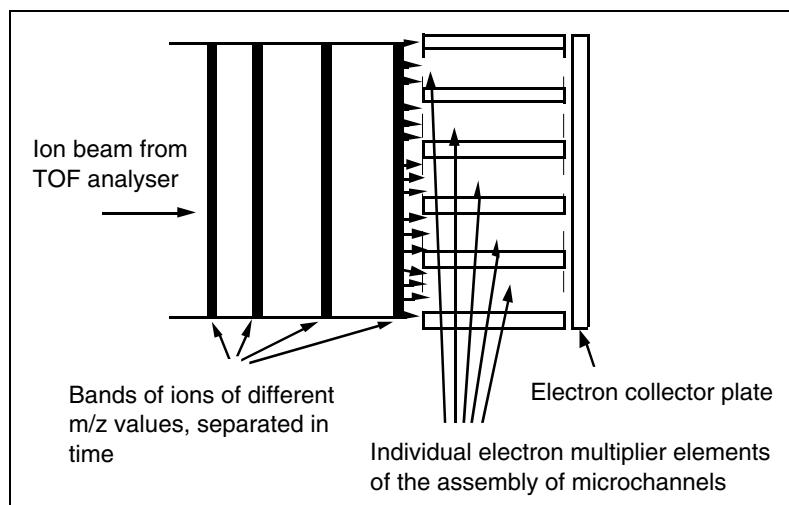


Figure 5 Bands of ions of different m/z values and separated in time in a broad ion beam travelling from left to right towards the front face of a microchannel assembly. The ions produce showers of electrons and these are detected at the collector plate, which joins all the elements in one.

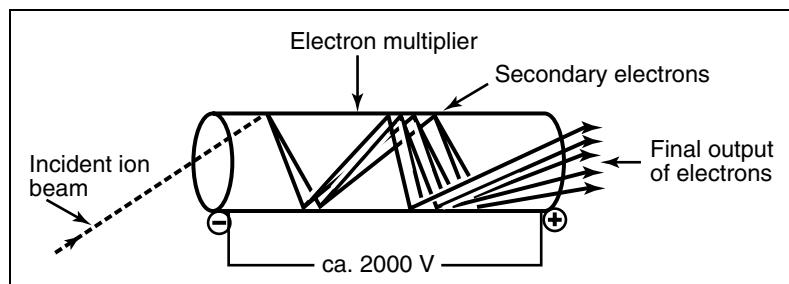


Figure 6 A typical single microchannel electron multiplier. Note how the primary ion beam causes a shower of electrons to form. The shower is accelerated towards the other end of the microchannel, causing the formation of more and more secondary electrons.

The elements of array and microchannel plates

The Back-to-Basics guides to Single Point and Array Collectors should be consulted for greater detail on how they work. Where space is not a problem, a linear electron multiplier having separate dynodes to collect and amplify the electron current created each time an ion enters its open end can be used. For array detection, the individual electron multipliers must be very small so that they can be packed side-by-side into as small a space as possible. For this reason, the design of an element of an array is significantly different from that of a standard electron multiplier used for point ion collection, even though its method of working is very similar. Figure 6 shows an electron multiplier (also known as a channeltron[®]) that works without using separate dynodes. Because each channeltron can be made very small, it is suited to array assemblages, in which as many multipliers as possible can be fitted side by side into a relatively small space. Each channeltron element can be made in a variety of shapes, one of which is shown in Figure 7.

For a microchannel plate, the back end of each element is left open as shown in Figure 5 or 6 and forms a 'microchannel'. Any electrons emerging from whichever element are all detected by the one collector plate.

Array elements (ion mass range)

Consider just two array elements of the ones illustrated in Figure 4 and suppose an ion beam has been dispersed to give ions of m/z values 100 and 101. If the dispersion is correct for the array size, the ion of m/z 100 will enter one element and, at the same time, the ion of m/z value 101 will enter a second adjacent one. Thus, at this level of dispersion, unit m/z values can be separated. Simple extrapolation to say 5 ions of different m/z values or 10 array elements in a line shows that several ion m/z values and abundances can be measured simultaneously to give an instantaneous spectrum. Further extrapolation indicates that more m/z values can be measured if there are more array elements. However, these do not come without cost and fitting a very large number of elements into a compact array becomes increasingly difficult. Therefore, a limited number of array elements is used, say 100, which means that sections of a mass spectrum can be measured instantaneously but, if it is required to measure a spectrum spread over several hundred or thousand mass units then it must be measured in sections at a time. Frequently, not all of a mass spectrum is needed and it is may be unnecessary to measure more than one region of the spectrum, e.g., maybe only the molecular ion region needs to be covered.

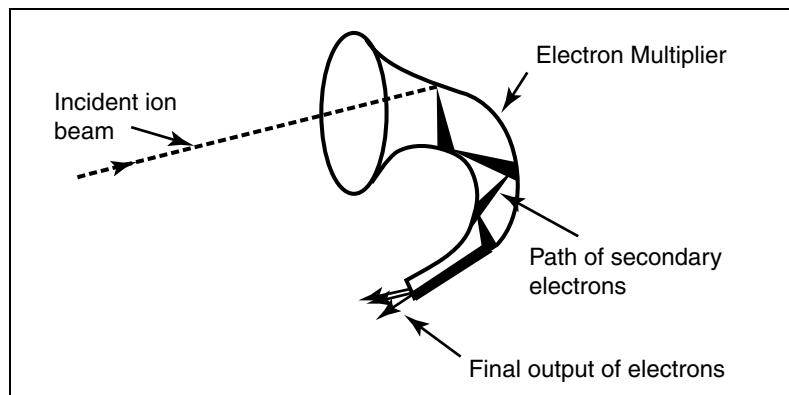


Figure 7 A different form of miniature electron multiplier. The curved shape is used to reduce back-scattering of the electrons. The final output of electrons flows along a wire to an amplifier.

**Microchannel elements
(ion mass range)**

Consider again two detection elements and suppose an ion beam has been dispersed in time such that ions of m/z 100 arrive at each of several elements (Figure 5). In this time-of-flight mode, the next ion of m/z 101 has not yet arrived and the ion of m/z 99 has arrived previously. Although the m/z ions are dispersed in time over a region of space and strike different elements of the detector, they are collected and monitored simultaneously because all of the microchannels are electronically connected. The operation of the microchannel plate is much easier than is the array because all the elements are monitored as one in the plate but each element must be monitored separately in the array. The microchannel plate detector is tremendously useful for those cases in which ions at each m/z value are separated in time by a mass analyser but may be delivered to the collector spread out in space. No ions are lost through scanning and the microchannel plate forms a very sensitive detector.

Uses of array and microchannel collectors**(a) Array collectors**

The major advantage of array detectors over point ion detectors lies in their ability to measure both a range of m/z values and the corresponding ion abundances all at one time, rather than sequentially. For example, suppose it takes 10 milliseconds to measure one m/z value and the associated number of ions (abundance). To measure 100 such ions of different m/z values with a point ion detector would require 1000 milliseconds (1 second). For the array detector, the time is still only 10 milliseconds because all the ions of different m/z values arrive at the collector at the same time. Therefore, when it is important to be able to measure a range of ion m/z values in a short space of time, the array detector is advantageous. There are two common occasions when instantaneous measurement of a range of m/z values is preferable. First, with ionization sources such as those using laser desorption or radionuclides, a pulse of ions is produced in a very short interval of time, often of the order of a few nanoseconds. If the mass spectrometer takes 1 second to attempt to scan the range of ions produced then, clearly, there will be no ions left by the time the scan has completed more than a few microseconds (ion traps excluded). The array collector overcomes this difficulty by detecting the ions produced all at the same instant.

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A second use of arrays arises in the detection of trace components of material introduced into a mass spectrometer. For such very small quantities, it may well be that, by the time a scan has been carried out by a mass spectrometer having a point ion collector, the tiny amount of substance may have disappeared before the scan has been completed. An array collector overcomes this problem. Often, the problem of detecting trace amounts of a substance using a point ion collector is overcome by measuring not the whole mass spectrum but only one 'characteristic' m/z value (single ion monitoring or single ion detection). However, unlike array detection, this single ion detection method does not provide the whole spectrum and an identification based on only one m/z value may well be open to misinterpretation and error.

(b) Microchannel plate collectors

A major advantage of microchannel plate detectors over point ion detectors lies in their ability to measure the abundance of ions of a single m/z value, which are spread over a region of space. When used with a TOF analyser, another major advantage appears. Typically, in a TOF instrument, ions of adjacent m/z values in the ion beam are separated by about 20 to 30 nanoseconds. Over a total mass range of 0-3000 mass units, all of the ions arrive at the collector within a period of about 30 microseconds. Therefore, the microchannel plate acquires a mass spectrum in about 30 microseconds, which on the human time scale appears to be instantaneous. Thus, like the array detector on a magnetic scanning instrument, the microchannel plate on a time-of-flight instrument is capable of generating an almost instantaneous spectrum. Additionally, a full range of ions from, say, 0 to 3000 mass units may be pulsed into a TOF analyser at the rate of about 30,000 times per second. The microchannel plate acquires all of these spectra, which can be summed. Thus, in one second, a TOF/microchannel plate combination can sum about 30,000 spectra. This is a great advantage for examination of spectra, which contain spurious occasional electronic 'noise' or which have been generated in a small period of time, as with laser-assisted ionization. Other uses of the microchannel plate are described in the Back-to Basics guide titled Orthogonal TOF.

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Conclusion

A multipoint ion collector (also called the detector) consists of a large number of miniature electron multiplier elements assembled, or constructed, side by side over a plane. A multipoint collector may be an array, which detects a dispersed beam of ions simultaneously over a range of m/z values and is frequently used with a sector type mass spectrometer. Alternatively, a microchannel plate collector detects all ions of one m/z value. When combined with a time-of-flight analyser, the microchannel plate affords an almost instantaneous mass spectrum. Because of their construction and operation, microchannel plate detectors are cheaper to fit and maintain. Multipoint detectors are particularly useful for situations, in which ionization occurs in a very short space of time, as with some ionization sources, or in which only trace quantities of any substance are available. For such fleeting availability of ions, only multipoint collectors can measure a whole spectrum or part of a spectrum satisfactorily, in the time available.

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Back to Basics Section E: Ion Collectors

CHAPTER E4

TIME-TO-DIGITAL CONVERTERS (TDC)

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Quick Guide

- In Time-of-flight mass spectrometers, a pulse of ions is accelerated electrically at zero time. Having attained a maximum velocity, the ions drift along the flight tube of the analyser. The times of arrival of ions at a detector are noted.
- From the flight times, it is easy to deduce the m/z values for the ions and then to produce a mass spectrum.
- The times taken for the ions to drift along a typical analyser tube are only a few microseconds and a very accurate 'clock' is needed if the derived m/z values are to be accurate.
- This timing is done electronically by making use of a microchannel array ion collector and 'time bins'. The microchannel array sends an electrical pulse to a time bin. Since a pulse is recorded, the ion arrival times are already digitised and hence the name, Time-to-Digital Converter (TDC).
- Basically, a series of consecutive time bins covers a length of time of a few milliseconds, with each bin representing a time of only a fraction of a nanosecond. When an ion arrives at the microchannel array detector, one time bin notes the resulting electronic pulse.
- By observing which bin in the series has been affected by the pulse from an ion arrival event and knowing how much time each bin represents, it is an easy matter to find the time taken for the ion to drift the length of the analyser tube.
- For example, if each bin represents 0.3 ns and bin number 200 has been affected by an ion arrival, then the flight time must have been $200 \times 0.3 = 60$ ns. Knowing the length of the drift tube, the ion drift velocity can be calculated and, from that, its m/z value may be deduced.
- Time bins allow very small time intervals to be measured accurately and therefore provide accurate m/z values.
- One drawback of time bins relates to the events they record. If two or more ions arrive at the array detector at the same instant, the resulting electrical pulse is the same as if only one ion had arrived. The bins are 'blind' to multiple concurrent events.
- This problem is known as 'dead time'. To offset this effect, an algorithm is used to adjust the actual number of events into a 'true' number of events. Since the numbers of ions represent ion abundances, the correction adjusts only abundances of ions before a mass spectrum is printed.
- Ion arrival times are not adjusted and the observed m/z values are just those originally measured.

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Summary By making use of electronic means for measuring very small time intervals, accurate m/z values can be measured on time-of-flight mass spectrometers. Ion arrival times represent pulses of electricity (digits) and do not need to be converted from analogue signals into digitised ones, hence the name Time-to-Digital Converter.

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TIME-TO-DIGITAL CONVERTERS

Background

Point and array ion collectors have been described (Back-to-Basics). A development of the array has been the multipoint ion collector, in which many thousands of very small diameter ‘microchannels’ are packed closely together. This arrangement gives a large number of ion collectors within an area large enough to encompass the cross-sectional area of a typical ion beam in a mass spectrometer. Unlike the array collectors, in which each ‘chaneltron’ is separately monitored so as to distinguish between m/z values, the microchannel plates simply record total ion arrivals and are particularly suited to time-of-flight analysers. Wherever an ion arrives at the front face of the microchannel plate, it results in a release of electrons to a positively charged electrode (back plate). The resulting electric current is passed to a suitable recording device. Ions in a time-of-flight analyser arrive at the ion collector spread over a period of time; the arrival events (pulses) are recorded. These electrical signals are already digitised. Since the collector takes ion arrivals spread over time and converts these events into discrete (digital) electrical pulses, it is called a time-to-digital converter (TDC). A more detailed description of the operation of a TDC appears below.

Measurement of m/z ratios by time-of-flight instruments

This topic is covered in detail in the Back-to-Basics guide *TOF Ion Optics*. Briefly, after acceleration through an electric potential difference of V volts, ions reach a velocity v governed by the equation, $v = (2zeV/m)^{0.5}$, in which e is the electronic charge. Note that the velocity is inversely proportional to the m/z value of an ion. If the ions travel a distance d before being detected, the time t needed to travel along the TOF flight tube is given by $t = d/v$ and m/z becomes proportional to t^2 (Figure 1). Thus, mass measurement resolves itself into the accurate measurement of the times needed for ions to travel along the flight tube. This timing must be done electronically because ‘normal’ clocks cannot measure the times accurately enough for flight tubes of reasonable length (say, 1.0 metre). As an example, for a potential difference of 1000 volts, a mass of 100 and a charge of $z = 1$, ions would take 22.8 μs to travel the length of the TOF flight tube and a mass of 101 would take 22.9 μs . If mass 100 is to be differentiated (resolved) from 101 then the timer must be able to accurately measure time differences of at least 0.1 μs . Time-to-digital converters (TDC) can effect such accurate time measurement and provide a very

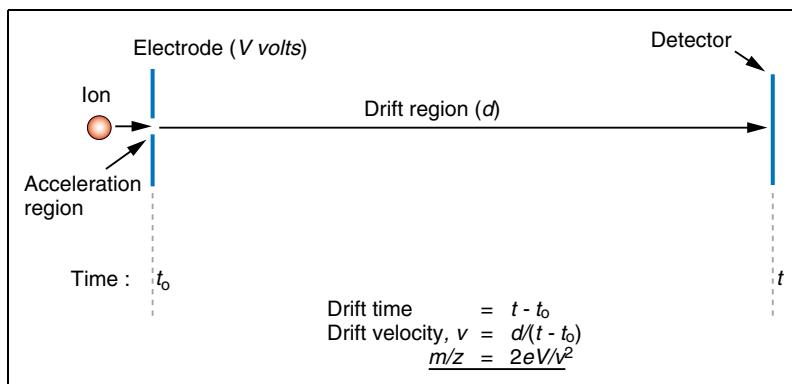


Figure I On acceleration through an electric potential of V volts, ions of unknown m/z value reach a velocity v ($= [2eV/m]^{0.5}$). The ions continue at this velocity (drift) until they reach the detector. Since the start (t_0) and end (t) times are known, as is the length d of the drift region, the velocity can be calculated and hence the m/z value can be calculated. In practice, an accurate measure of the distance d is not needed because it can be found by using ions of known m/z value to calibrate the system. Accurate measurement of the ion drift time is crucial.

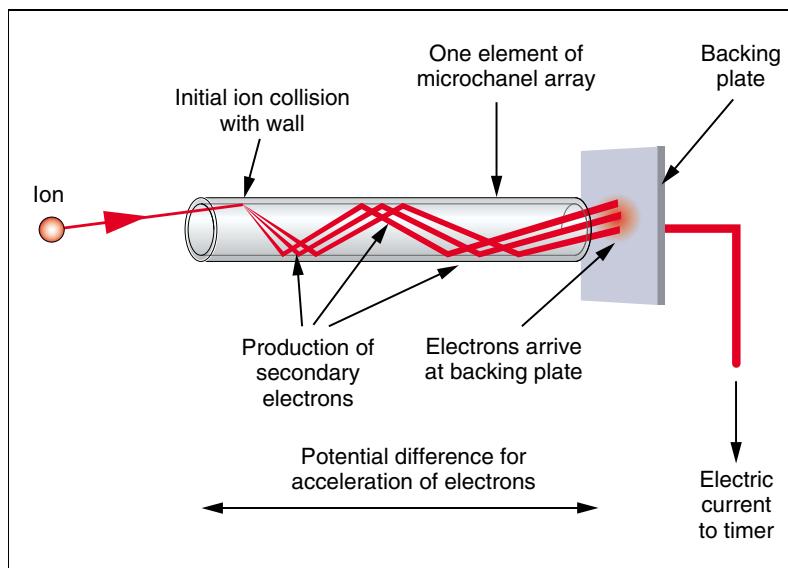
convenient method of recording mass spectra from a TOF instrument, particularly as the signal coming from the converter is not analogue but is already digitised.

Ions in a TOF analyser are temporally separated according to mass. Thus, at the detector all ions of one mass arrive at one time and all ions of another mass arrive at a different time. Apart from measuring times of arrival, the TDC device must be able to measure the numbers of ions at any one m/z value so as to obtain ion abundances. Generally, in TOF instruments, many pulses of ions are sent to the detector per second. It is not unusual to record something like 30,000 spectra per minute. Of course, each spectrum contains few ions and a final mass spectrum requires addition of all 30,000 spectra so as to obtain a representative result.

Multichannel (microchannel) plate array

The mode of action of a single ‘chaneltron’ (a miniaturised version used as one element of a microchannel plate array) is shown in Figure 2. A fast moving ion striking the front end of a single chaneltron causes a number of secondary electrons to be ejected. These electrons are accelerated by an electric field lying along the length of the microchannel element and, after a short distance, strike its walls further along from their point of origin. Each electron impact on the chaneltron walls itself causes several electrons to be ejected; each initial secondary electron produces several more electrons. This process of producing more electrons continues along the length of the microchannel element, with further wall collisions leading to a cascade of many electrons, which emerge as a burst (pulse) from the other end of the microchannel. The pulse of electrons crosses to a positively charged metal ‘plate’ collector and is detected as an electrical current. Thus, each time an ion arrives, an electrical pulse (electrical digit) is produced. A succession of ion arrivals yields a corresponding series of electrical pulses. As noted above, these ion arrival events are already digitised and can be stored directly in ‘computer memory’ (see below). Thus, each ion event is transformed into an electrical digital pulse and this process gives rise to the term, time-to-digital converter (TDC).

The front opening of such a microchannel element has a diameter of only a few microns but it is only one element of a whole multichannel array (Figure 2). Whereas the orifice to one microchannel element covers an area of only a few square microns, an array of several thousand parallel elements covers a much larger area. In particular, the area covered by the array must be larger than the cross-sectional area of the ion beam that is being detected so that all ions are recorded. Figure 3 shows a small representative section of a multichannel array, together with the backing plate.

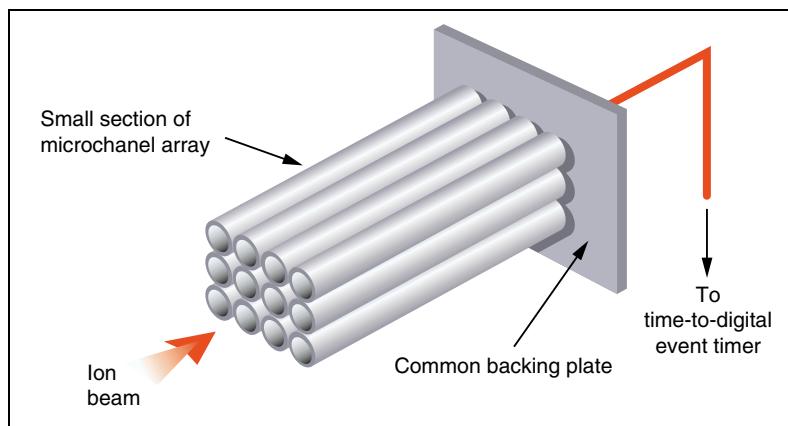
**Figure 2**

The diagram illustrates one element of a microchannel array. In a typical array, this 'chaneltron' like tube is just one of thousands of similar ones, each only a few microns in diameter. All microchannel elements end a short distance from a common backing plate. An ion entering the front end of an array element and striking the wall produces secondary electrons. These in turn are accelerated along the tube and strike the wall. Each secondary electron produces several more secondary electrons. The process is repeated along the tube until a shower of electrons leaves its end and crosses to the backing plate. The arrival of these electrons constitutes a pulse of electric current, which is recorded by the time-to-digital timer. The ion arrival time (an event) is recorded.

Timing of electrical pulses resulting from ion arrivals at the microchannel plate collector

As shown above, when an ion arrives at the microchannel array it releases a cascade of electrons onto the ‘back plate’. This constitutes an electrical pulse from the microchannel plate, which is used for timing ion arrival events. Electronically, each pulse resulting from an ion arrival travels to and is recorded by a ‘time bin’. A series of connected time bins may be regarded as a long chain of electronic ‘gates’, through which a clock signal passes. The time for the signal to pass through each gate is about 3×10^{-10} seconds (0.3 ns). Thus, as shown in Figure 4, if a gate timing pulse is regarded as having started at time zero ($t = 0$) then the time taken for the pulse to pass through 100 bins is 30 ns ($t_{100} = 3 \times 10^{-8}$ seconds and $t_{100} - t_0 = 3 \times 10^{-8}$ seconds). Any one bin can be electronically distinguished from the next one and therefore the bins can be used like the ‘tick’ of a standard clock. Each bin serves as one tick, which lasts for only 0.3 ns. By ‘counting’ the ticks and knowing into which bin the ion pulse has gone, then the time taken for the ion to arrive at the detector can be measured to an accuracy of 0.3 ns. This is the basis for measuring very short ion arrival times after the ions have travelled along the TOF analyser tube. Each ion arrival pulse (event) is extracted from its time bin and stored in an associated computer memory location.

An explanation of the scheme for storage of the event signals requires an extra degree of complexity. The bins have two states (‘on’ or ‘off’, digitally 0 or 1). Before the timing pulse begins, all bins are set to the 0 state, which changes to a 1 state when the timing pulse arrives. When an ion arrival event occurs, the clock timing signal will have already travelled through some of the bins, *viz.*, it will have passed through some of them in sequence, setting their states to from 0 to 1. When the ion signal (a ‘hit’) arrives, it is sent to all of the time bins simultaneously and the bins will be affected in different ways (Figure 4). Those bins through which the timing signal has already passed will have been reset to the ‘1’ state but, immediately in front of the timing signal, the next bin will still be set to the original ‘0’ state. Those bins already affected by the timing signal will be set back to 0 when the ‘hit’ signal arrives. Thus, all of the bins up to the event signal will be in the 0 state, but those after the event signal will be in the 1 state. After the timing signal has passed right through all of the bins, they are examined. All those bins that have not recorded a ‘hit’ are regarded as ‘empty’ but the first bin that was set to 1 by a combination of hit and timing pulses must be the one into which the ‘hit’ signal went. Thus, an electronic ‘inspection’ of the bins reveals that the ‘0/1’ state of the bins changes at one of them and this is stored in permanent memory. Since the position of any bin in the series is known relative to the starting bin, the time at which the ‘hit’ signal from an ion arrival affected the bins can be measured to an accuracy

**Figure 3**

Diagrammatic representation of a small section of a microchannel array, with its backing plate. One element of the array is shown in greater detail in Figure 2. Arriving ions may enter any one of the array elements to produce an electric current at the backing plate. Since the latter is common to all of the array elements, it is immaterial which element of the array is involved because the signal is recorded by the common plate. Thus, arrival of an ion at any point on the array (an event) results in an electrical signal being sent to the time-to-digital timer (TDC converter). This is the ion arrival time (t) shown in Figure 1.

of about 3×10^{-10} seconds. After the event has been noted, all bins are reset to their '0' states before the next timing signal travels along them ready for the next ion arrival. A typical clock timer runs at about 3.6 GHz, viz., it 'ticks' every 0.28×10^{-9} seconds (every 0.28 ns).

In a simplistic way, the bins may be regarded as a series of empty receptacles and that an ion arrival (an event) results in one bin being filled. By 'looking' into the line of bins, the full one can be found. Rather than discussing tiny fractions of a second, let it be supposed that it takes 1 second to pass from one bin to the next in a series of 1000 bins. The total time needed to pass through them all is 1000 seconds. If only bin number 603 is found to be full, then the timing of the event that filled it must have occurred 603 seconds after the examination began. All empty bins are ignored but the full bin is emptied and noted. Thus, the bins are returned to their original empty state, after which the timing begins all over again in preparation for the next ion event.

Each bin is connected to a memory location in a computer so that each event can be stored additively over a period of time. All the totalled events are used to produce a histogram, which records ion event times versus the number of times any one event occurs (Figure 5). With a sufficiently large number of events, these histograms can be rounded to give peaks, representing ion m/z values (from the arrival times) and ion abundances (from the number of events).

As noted above, for time-of-flight instruments, ion arrival times translate into m/z values and, therefore, the time and abundance chart becomes mathematically an m/z and abundance chart, viz., a normal mass spectrum is produced.

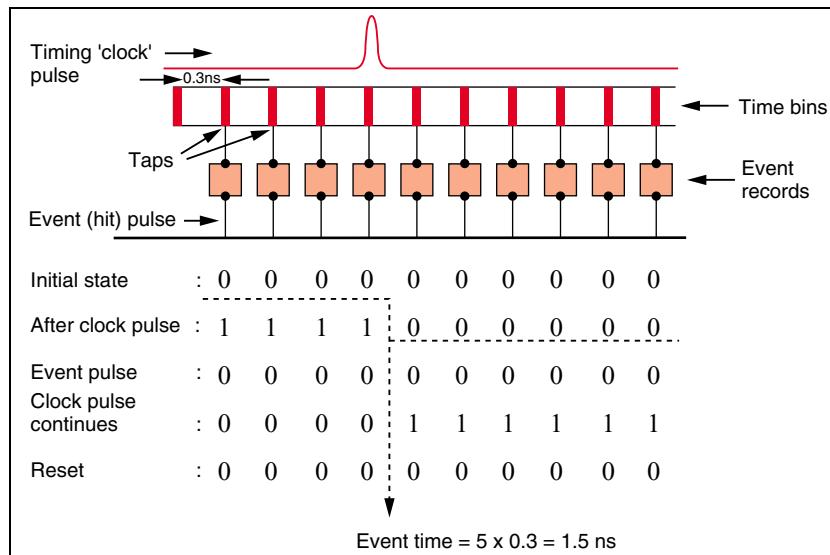


Figure 4 A ‘clock’ pulse travels along a series of electronic ‘gates’ (time bins). The time taken to cross each gate is about 0.3 ns. Passage of the pulse through a gate is recorded by ‘tapping’ a part of the electrical signal from the clock pulse, viz., part of the signal is sent to the event recorders, which switch from ‘0’ to ‘1’ states. In this example, after passing through 5 gates, the elapsed time from the start of the clock pulse is 1.5 ns. Arrival of an ion (an ‘event’ or ‘bit’) causes a new pulse to be sent to *all* of the event recorders. This resets those affected by the clock pulse. The clock pulse continues to travel along the time bins, changing the event recorders from ‘0’ to ‘1’. Where the event has occurred is marked by a change of state of the event recorders. Before the timers are reset, the recorded event is sent to a more permanent memory store for later use.

**Ion abundances and
'dead time'**

A mass spectrum is a chart of ion abundances versus m/z values. It has been shown above that the TDC measures ion arrival times, which are converted directly into m/z values. Notionally, the number of ions arriving at the detector at any one m/z value is equal to the number of events recorded (one ion in, one event recorded). With a recording time of only a few microseconds, there will not be many ions arriving together at the detector at exactly the same time, even when the ions are abundant. However, the more abundant the ions, the less likely it becomes that the relationship of one ion, one event will be true. Suppose for abundant ions at m/z 100 that, in one recording, two ions arrive at the microchannel plate array at the same time, even in different elements of the array. As shown above, the TDC system cannot differentiate between one or two or more ions arriving at the same time (within a time bin time of 0.3 ns). Therefore, abundant ions are under-recorded. The difference between the true numbers of ions and the numbers suggested by the events counter can be substantial. The time bins are 'blind' to multiple events occurring within a space of about 0.3 ns. This is known as 'dead time' and must be corrected to give a more accurate assessment of true ion abundance. A mathematical algorithm is used to increase the apparent ion yield to a 'true' ion yield before the mass spectrum is printed. The algorithm increases the number of events in proportion to the numbers of events recorded. Many events at one m/z value indicate that the correction needs to be larger and vice versa. Figure 6 illustrates the effect on ion abundances of the correction for dead time.

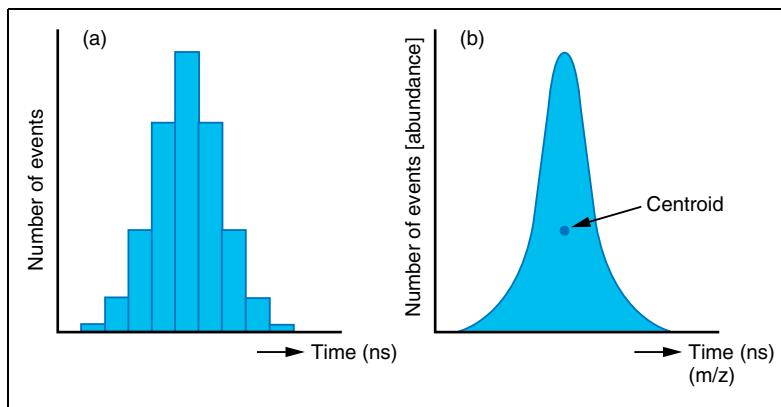


Figure 5 Ion arrival events are stored additively in memory locations (Figure 4), which also provide the times of arrival. After recording data for some time, the memories are examined to produce a histogram (a) of the number of events versus arrival times. The histogram shown relates to ions of one particular m/z value arriving at the detector at slightly different times. From the histogram, a peak shape is produced (b), the centroid of which gives the mean arrival time and, therefore, the m/z value (Figure 1). The area of the peak gives the total number of events and therefore the abundance of the ions. Similar procedures are used for all other ions of other m/z values in the mass spectrum

Conclusion Time-to-digital converters are very convenient for measuring the times of arrival at a detector of ions in a TOF analyser. The signal resulting from the detection of each ion is already digitised and no analogue-to-digital converter is needed prior to the signals being evaluated by a computer. Before conversion to a mass spectrum, abundances of ions must be corrected for dead time.

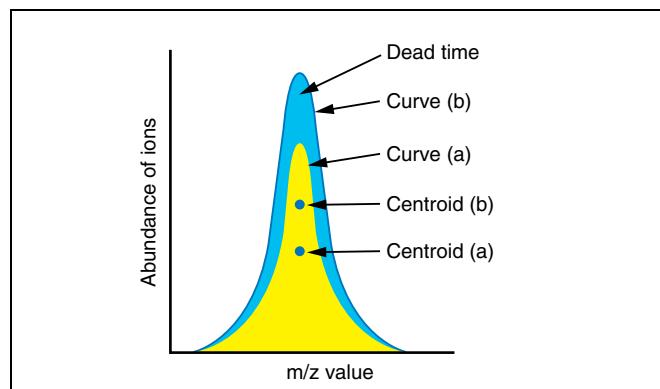


Figure 6 The curve (a) traces the outline of the peak obtained directly from the number of events recorded (Figure 5). The second curve (b) traces the outline of the peak obtained after correcting for coincidental events ('dead time', shown by the shaded area). The centroids of peaks (a,b) are shown and it can be seen that they occur at the same m/z value, *viz.*, the dead time correction alters only the abundances and not the m/z values of the ions.

**Back to Basics Section F:
Special Ion Manipulation Methods**

CHAPTER F1

**ORIGIN AND USES OF METASTABLE
IONS**

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Quick Guide

- Metastable ions are most readily detected following electron ionization. For other ionization methods, the Guide on 'Induced Dissociation' should be consulted.
- In the few microseconds that molecular ions (M^+) spend in an ion source following electron ionization, many have sufficient energy to decompose to give fragment ions ($F_1^+, F_2^+, \dots, F_n^+$).
- This assembly of ions (M^+, F_1^+, \dots, F_n^+) in the source is analysed by the mass spectrometer to give a mass (m/z) versus ion abundance chart, i.e., a mass spectrum.
- Ions formed in the source in this way compose the majority and are called 'normal' ions.
- A small proportion of the ions formed in the source have insufficient energy to fragment there but have just sufficient energy to decompose in the few microseconds of flight between the source and the detector. These are the 'metastable' ions.
- Modern mass spectrometers are set to transmit and measure normal ions but, under normal circumstances, the metastable ones are not recorded.
- By adjusting magnetic and electric fields in the ion optics (linked scanning - see Guide), metastable ions can be investigated.
- Normal ions (M^+, F_1^+, \dots, F_n^+) in a spectrum can provide a molecular structure for substance (M) if the fragments can be theoretically reassembled. The problem is rather like deducing an original jigsaw picture by putting the pieces together correctly. For most molecules containing more than a few atoms this reassembly exercise is difficult and often problematic.
- To carry out the reassembly exercise, connectivities between the ions must be determined. For example, does the ion, F_3^+ , come from M^+ or F_1^+ or F_2^+ ? This process can be likened to completing a jigsaw by finding where and how the pieces fit together.
- In mass spectrometry, the required connectivities (links) can be obtained from observations on metastable ions. For example, finding a metastable ion for a supposed process, $M^+ \rightarrow F_3^+$, proves the process exists.

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- By measuring a mass spectrum of normal ions and then finding the links between ions from the metastables it becomes easier to deduce the molecular structure of the substance which was ionized originally.

Summary Metastable ions are useful for determining the paths by which molecular ions of an unknown substance have decomposed to give fragment ions. By ‘retracing’ these fragmentation routes, it is often possible to deduce the molecular structure of the unknown.

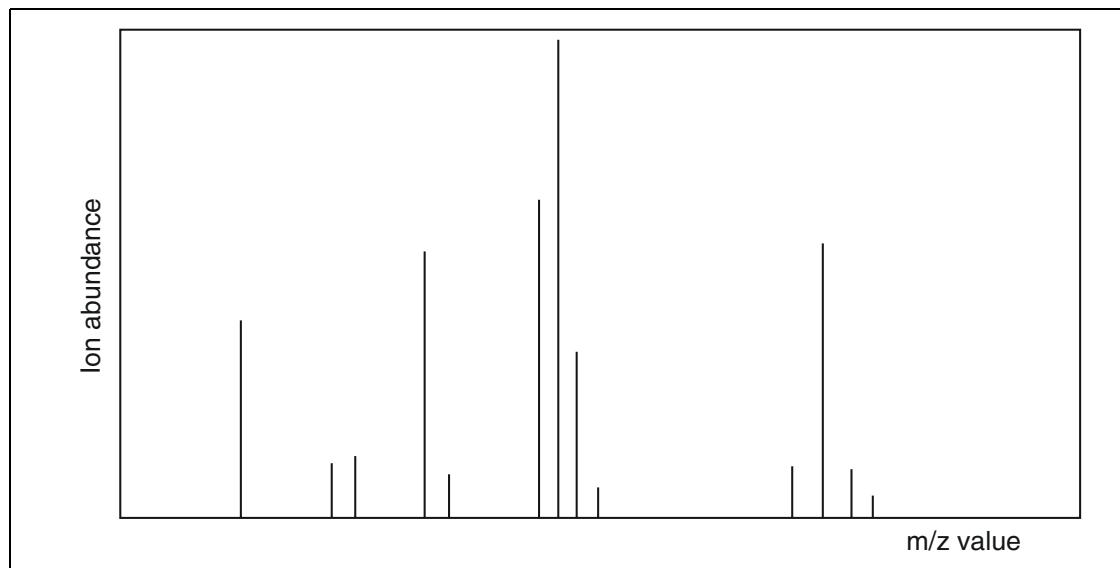


Figure 1 An example of a mass spectrum showing the peaks (or lines) corresponding to ions measured at various m/z values by the spectrometer; the heights of the peaks relate to the abundances of the ions.

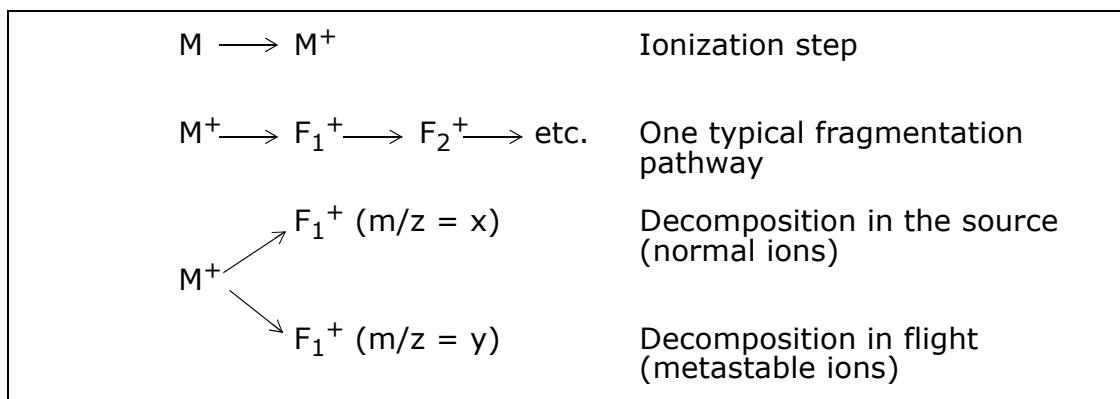


Figure 2 The first ionization step in the source converts neutral molecules (M) into ions (M^+). After a short time in the source, some molecular ions have fragmented to give smaller ions, F_1^+ , F_2^+ ... F_n^+ , along one or more reactions pathways. An ion (F_1^+) formed in the source and then analysed will have an $m/z = x$; the same ion (F_1^+) formed outside the source will have an *apparent* $m/z = y$, where $y < x$.

ORIGIN AND USES OF METASTABLE IONS

Introduction A mass spectrum consists of peaks corresponding to ions. The position of a peak on the x-axis is proportional to its mass (strictly m/z value), whilst the height of the peak on the y-axis gives the number of ions (abundances) at a particular m/z. The ions giving rise to the spectrum are formed in an *ion source* and are passed through an *analyser* for measurement of m/z and into a *detector* for measurement of abundance (Figure 1).

From molecules of a substance (M), electron ionization gives first the molecular ions (M^+) which remain in the ion source for several microseconds before travelling through the instrument to the detector. During their time in the source some or many of the molecular ions which possess sufficient energy will decompose to give fragment ions, F_1^+ , F_2^+ F_n^+ (Figure 2) along various reaction (fragmentation) pathways; the fragment ions themselves decompose to other fragments until all excess of energy is used up. The mass spectrometer draws out (samples) the assembly of ions (M^+ , F_1^+ , F_2^+) in the source and measures m/z and abundance values for each species to provide a spectrum of 'normal' ions, viz., a spectrum of the ions in the source. For example, as shown in Figure 2 normal ions (F_1^+) will have m/z = x.

However, a small proportion of ions will have insufficient energy to fragment during the few microseconds spent in the source but will have just sufficient energy to fragment during the few microseconds of flight through the analyser to the detector. Such ions, which start out with a certain kinetic energy on leaving the source must lose some of that energy on decomposing (conservation of momentum between the fragments). Therefore, as far as the spectrometer is concerned, these ions have the 'wrong' kinetic energy for their mass which will be measured as m/z = y (Figure 2; $x > y$); these are the so called *metastable ions*. It is important to understand that the mass (m) of a normal fragment ion (F_1^+) is exactly the same as the mass (m) of the corresponding metastable ion (F_1^+). The only difference between them lies in their positions of formation (inside or outside the source). The spectrometer correctly measures m/z for F_1^+ (normal) ions formed in the source but gets the "wrong" m/z for F_1^+ (metastable) ions formed after the source. In other words, although normal and metastable F_1^+ ions have the same mass, the mass spectrometer interprets their m/z values differently because normal F_1^+ ions have greater momentum than the metastable ones.

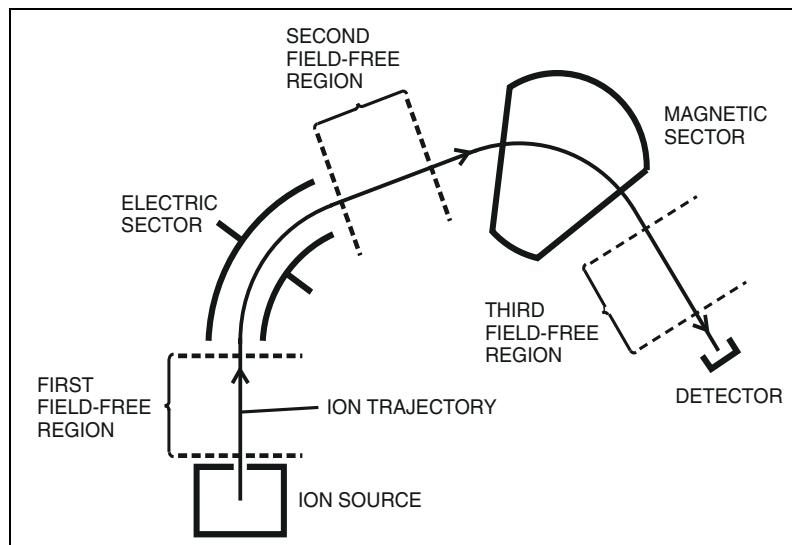


Figure 3 Ion trajectory through a conventional (EB) sector instrument, showing three field-free regions in relation to the sectors, the source and the ion detector.

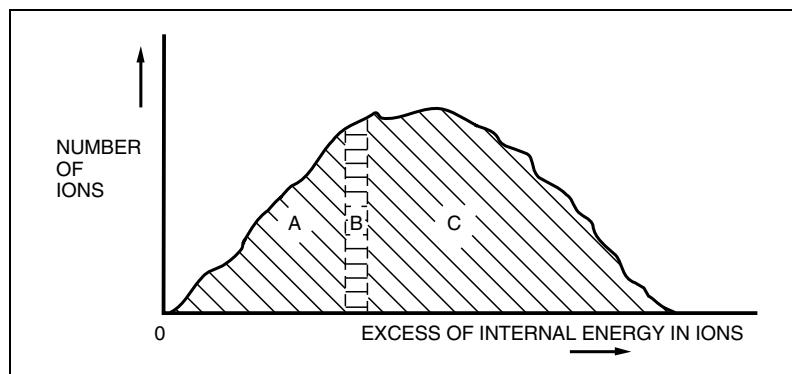


Figure 4 A correlation between the number of ions and their excess of energy. In region A, the molecular ions have insufficient energy to fragment but, in region C, they have enough to cause decomposition in the ion source. Ions metastable in the narrow band, B, have just the amount of excess to cause fragmentation in flight.

However, the spectrometer can be set to record the metastable ions correctly and not the normal ones. The required settings of the spectrometer to observe metastable ion processes form the subject of the Guides on 'Linked Scanning'.

Perhaps it is worth emphasising that the actual metastable ions are those that decompose and the so called metastable ions that are recorded are actually the products of decomposition, not the metastable ions themselves. It is more accurate to describe the recorded "metastable" ions as ions resulting from metastable ions decomposition.

Field Free Zones and The Formation of Metastable Ions

Certain regions of a mass spectrometer have no electric or magnetic fields to affect an ion trajectory (field-free regions). Figure 3 illustrates three such regions in a conventional double-focusing instrument. Metastable ions formed in any of these field-free regions can be detected by specialized changes in the various electric and/or magnetic fields (linked scanning), without interference from normal ions formed in the source. In a one-sector magnetic instrument there are only two field-free regions. In tandem MS, there is always one more possible field-free region than there are sectors; for example, a four-sector instrument has five such regions although, in practice, only one or two are used for metastable ion observations. In a simple quadrupole mass spectrometer, differentiation between normal and metastable ions is not possible but can be carried out efficiently with a triple quadrupole instrument (see Guide on Metastable Ions in Quadrupole Mass Spectrometry).

Abundances of Metastable Ions

When molecules are ionized, particularly by electrons (EI), an excess of energy is usually left in the resulting molecular ions. The spread in excess of energy can be represented generally as shown in Figure 4. Only that small proportion of ions (metastable) indicated as region B (Figure 4) has just such an excess of energy that fragmentation is delayed and takes place not in the ion source but during the few microseconds flight time between ion source and detector. Ions with excesses of energy falling within regions A and C give rise to the normal molecular and fragment ions observed in a mass spectrum. In region C, all of the excess of energy is used up in decomposition in the source whilst in A there is not enough energy for decomposition. Only in the small region B have ions just the right energy to fragment in flight. The resulting relatively low abundance of metastable ions makes their detection more difficult.

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Disadvantage of Low Abundance of Metastable Ions

The normal fragment ions in a mass spectrum potentially contain a wealth of information. Just as in a jigsaw puzzle, if the fragments (pieces) can be reassembled correctly, the original molecular structure (jigsaw picture) can be deduced (completed). Whereas in a jigsaw the relative positions of the pieces can be inferred from the way they interlock with neighbouring pieces (shapes), in a conventional mass spectrum, connectivities between *normal* fragment ions are not obvious and may be impossible to discover for large molecules. The molecular “jigsaw” has to be completed without seeing the shapes of pieces and being told only their masses. Because the metastable ions appear differently from normal ions in a mass spectrometer and yet form a link between precursor and product ions, a metastable spectrum (scan) provides the required connectivities between the normal ions. It is unfortunate that the relatively low abundances of metastable ions makes them difficult to observe.

Some mild methods of ionization (e.g., CI, FAB, ES) provide molecular or quasimolecular ions with so small an excess of energy that little or no fragmentation takes place. Thus, there are few, if any, normal fragment ions and metastable ones are virtually non-existent. Although these mild ionization techniques are ideal for yielding molecular mass information, they are almost useless for providing details of molecular structure, a decided disadvantage.

The absence or dearth of metastable ions could be counteracted if additional energy could be put into *normal* ions after they had left the ion source and whilst they were in a field-free zone. The additional energy could cause the normal ions to fragment in the field-free zone; this induced fragmentation is instrumentally indistinguishable from the decomposition giving rise to metastable ions as discussed above. Induced fragmentation increases the number of naturally-occurring metastable transitions occurring in the electron ionization process and can produce ‘metastable ions’ where none existed previously.

This enhanced fragmentation in field-free regions has led to big improvements in obtaining molecular structural information and in examining mixtures. It has produced the whole new technique of tandem MS (or MS/MS). Along with the enhanced fragmentation, a wide variety of means for examining the fragments and their origins has developed (linked scanning).

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Enhanced (induced) Fragmentation

To increase the excess of energy within an ion so as to induce it to decompose, a number of techniques are available. At the moment, only one of these has commercial importance, viz., collisionally induced decomposition or collisionally induced dissociation (CID)*. In this approach, normal ions *in flight within a field-free region* are given extra internal energy by causing them to collide with molecules of an inert gas, such as helium or argon. The collision takes place in a 'cell', a region of a field-free zone in which the inert gas pressure can be increased from zero to any desired value without decreasing the vacuum in the rest of the mass spectrometer. The collision process converts some of the kinetic energy of the ions into rotational, vibrational and electronic energy which, in turn, leads to fragmentation.

Collision of an ion with an inert gas molecule leads to some deflection in the ion trajectory. After several collisions, the ion could have been deflected so much that it no longer reaches the detector. This effect attenuates the ion beam as it passes through the gas cell, leading to loss of instrumental sensitivity. An attenuation of 50-70% is acceptable and is not unusual in practice.

Conclusion

Metastable ions yield valuable information on fragmentation in mass spectrometry such that an insight into molecular structure can be obtained. In electron ionization, metastable ions appear 'naturally' along with the much more abundant normal ions. Abundances of metastable ions can be enhanced by collision induced decomposition.

* This technique is also known as collisionally activated decomposition, CAD, but only the term CID, is used here.

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**Back to Basics Section F:
Special Ion Manipulation Methods**

CHAPTER F1.1

**LINKED SCANNING AND METASTABLE
IONS IN QUADRUPOLE MS**

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Quick Guide

- Ions formed in an ion source may be described as *normal* or *metastable*.
- Normal ions are readily and easily observed by quadrupole mass spectrometers.
- Metastable ions cannot be detected with simple quadrupole instruments.
- Knowledge of which normal ions in a mass spectrum fragment to which others is important and can be obtained from observations on metastable ions.
- Metastable ions can be detected efficiently by using three quadrupoles in tandem, a QQQ instrument.
- In use, the second quadrupole is set with its fields to pass all ions through to the third quadrupole. This set-up may be described as QqQ.
- By linking the way in which the first and third quadrupoles are scanned, specific metastable fragments can be detected. This is one form of linked scanning.
- Rather than look for just a low abundance metastable ion process occurring in the second quadrupole, extra fragmentation can be induced by having a neutral collision gas present in this quadrupole.
- Collision of normal ions from the first quadrupole with gas molecules in the second quadrupole leads to induced fragmentation and is known as either Collisionally Induced Dissociation (CID) or collisionally activated decomposition (CAD).
- Again, as for metastables, linked scanning of the first and third quadrupoles reveals important information on fragmentation processes viz., which normal ions fragment to give which others.
- Linked scanning provides important information about molecular structure, the complexities of mixtures, and the detection of trace components of mixtures.
- Linked scanning is particularly easy with a triple quadrupole instrument.

Summary

Triple quadrupole instruments can be used to detect metastable ions or can be used for linked scanning.

Note:- The guide on ‘Quadrupole Ion Optics’ should be read in conjunction with this one.

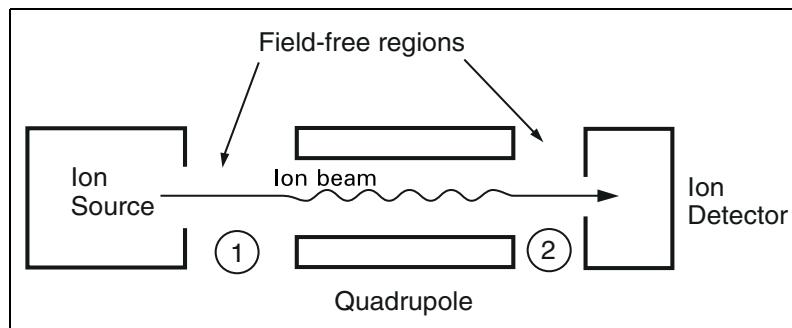


Figure 1 In a normal quadrupole instrument the field-free regions are very short. Ions formed in region (1) will be transmitted by the quadrupole as a normal ion. In region (2) there is no differentiation between metastable and normal ions.

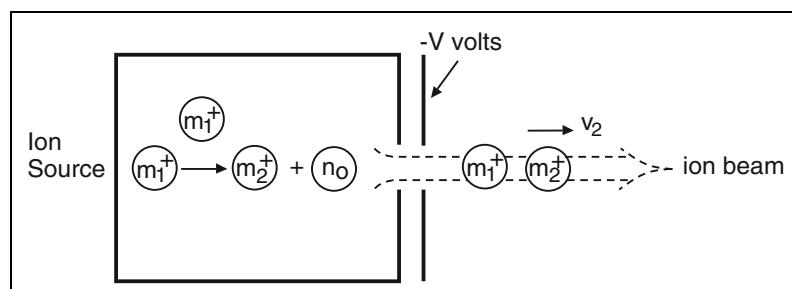


Figure 2 Formation of normal ions in an ion source. In this example, some initially formed ions (m_1^+) dissociate (fragment) to give smaller ions (m_2^+) and a neutral particles (n_o). Unchanged ions (m_1^+) and the fragment ions (m_2^+) are drawn out of the source as a 'beam', moving with velocities (v_1, v_2) respectively.

LINKED SCANNING AND METASTABLE IONS IN QUADRUPOLE MASS SPECTROSCOPY

Introduction The steps (reactions) by which normal ions fragment are important pieces of information, which are lacking in a normal mass spectrum. These steps can be deduced by observations on metastable ions and give important data on molecular structure, the complexities of mixtures and the presence of trace impurities. For ordinary quadrupole instruments (Figure 1), it is not possible to study metastable ions. The field-free regions are usually very short so that, in the time taken to traverse them, few ions dissociate. More importantly, a quadrupole is a mass filter. In the complex crossed DC and RF fields used for mass separation in a quadrupole assembly *there is no inherent force moving the ions along the central axis*, i.e., there is nothing to drive the ions through the length of the assembly. Ions can only traverse the length of the quadrupole if they have been previously given some momentum by acceleration through a (small) electric potential (about 5eV) on leaving the ion source. Unlike magnetic/electric sector instruments in which the forward motion of ions is used to help achieve mass separation, the forward momentum of an ion in a quadrupole instrument is very much less and effectively plays no such part, the mass separation being achieved by the crossed DC and RF fields at right angles to ion motion.

Fragmentation of Normal and Metastable Ions

Ions formed in the ion source itself are considered to be the normal ions in a mass spectrum. For example, as illustrated in Figure 2, some of the ions (m_1^+) dissociate to smaller mass ions (m_2^+) and neutrals (n_o); any unchanged ions (m_1^+) and the fragments (m_2^+) are drawn out of the source by an electric potential of V volts.

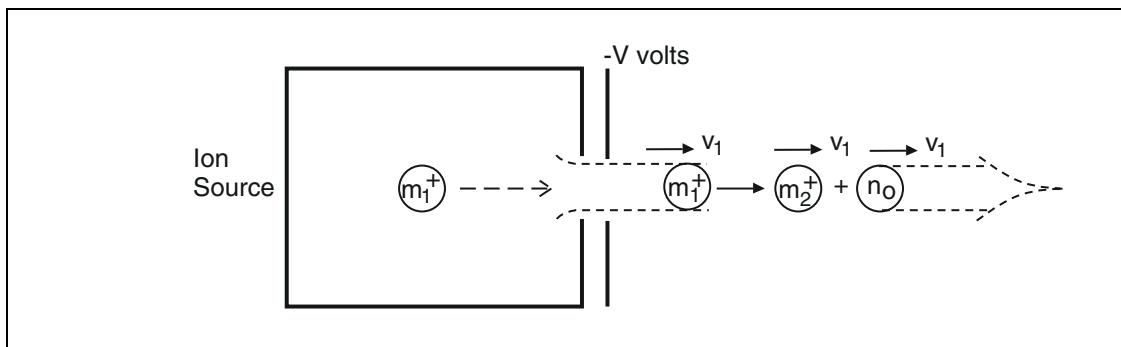


Figure 3 Ions (m_1^+) of velocity (v_1) which dissociate outside the ion source give ions (m_2^+) of the same velocity (v_1).

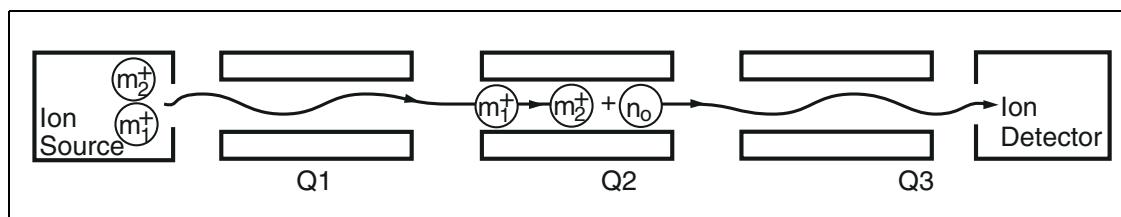


Figure 4 Normal ions (m_1^+ or m_2^+) formed in the ion source will pass (filter) through the first, second and third quadrupoles (Q1, 2, 3) if these are set correctly. If Q1 is set to pass only ions (m_1^+) then normal m_2^+ ions cannot reach the detector and, if Q3 is set to pass only ions (m_2^+) then m_1^+ cannot reach the detector. Ions (m_2^+) can only reach the detector if they have been formed (metastable) by dissociation of m_1^+ in Q2.

The kinetic or translational energy of the ions is equal to the work done on moving the charged species through the potential, V ,

$$\text{i.e. } \frac{1}{2}m_1v_1^2 = zV \text{ and, } \frac{1}{2}m_2v_2^2 = zV,$$

where z is the charge on the ions and v_1, v_2 are their final velocities.

$$\text{From this } v_1 = \sqrt{\frac{2zV}{m_1}} \text{ and } v_2 = \sqrt{\frac{2zV}{m_2}}$$

Now consider the same fragmentation process but, this time, the ion (m_1^+) dissociates outside the ion source.

$$\text{Initially, as for } m_2^+, \quad m_1v_1^2 = 2zV \text{ and } v_1 = \sqrt{\frac{2zV}{m_1}}.$$

When fragmentation occurs, the total kinetic energy of m_1^+ is shared between the new (metastable) ion m_2^+ and its accompanying neutral n_0 but their velocities remain unchanged (v_1). Normal m_2^+ ions formed in the source have velocity v_2 but metastable m_2^+ ions formed outside the source have the velocity of m_1^+ ($v_1; v_1 < v_2$; Figure 3). In a sector instrument, which acts as a combined mass/velocity filter, this difference in forward velocity is used to effect a separation of normal and metastable m_2^+ ions (see 'Ion Optics of Magnetic/Electric Sector MS'). However, as discussed above, the velocity difference is of no consequence to the quadrupole instrument which acts only as a mass filter and so normal and metastable m_2^+ ions formed in the first field-free region (figure 1) are not differentiated.

How Quadrupoles can be used to examine Metastable Ions

By using three successive in-line quadrupole assemblies, metastable ions can be examined (Figure 4). Ions from the source are mass separated in quadrupole Q1. For example, m_1^+ ions can be selected. The chosen ions are passed into quadrupole Q2 which has only RF voltages applied. In this mode, the ions from Q1 can pass through Q2 without further mass selection. If the m_1^+ ions dissociate in this region to give m_2^+ then these latter ions pass on into quadrupole Q3 which has its normal voltages applied for mass resolution. Thus, m_1^+ ions are selected by Q1 but rejected by Q3 and m_2^+ ions are rejected by Q1 but selected by Q3.

In this way, only metastable m_1^+ ions (passed by Q1 but rejected by Q2) which decompose to give m_2^+ in Q2 are detected in Q3. The triple quadrupole assembly is efficient for detection of metastable ions. The use of Q2 as an indiscriminate mass filter is sometimes indicated by the symbol, q, and the triple quadrupole may be referred to as QQQ or QqQ.

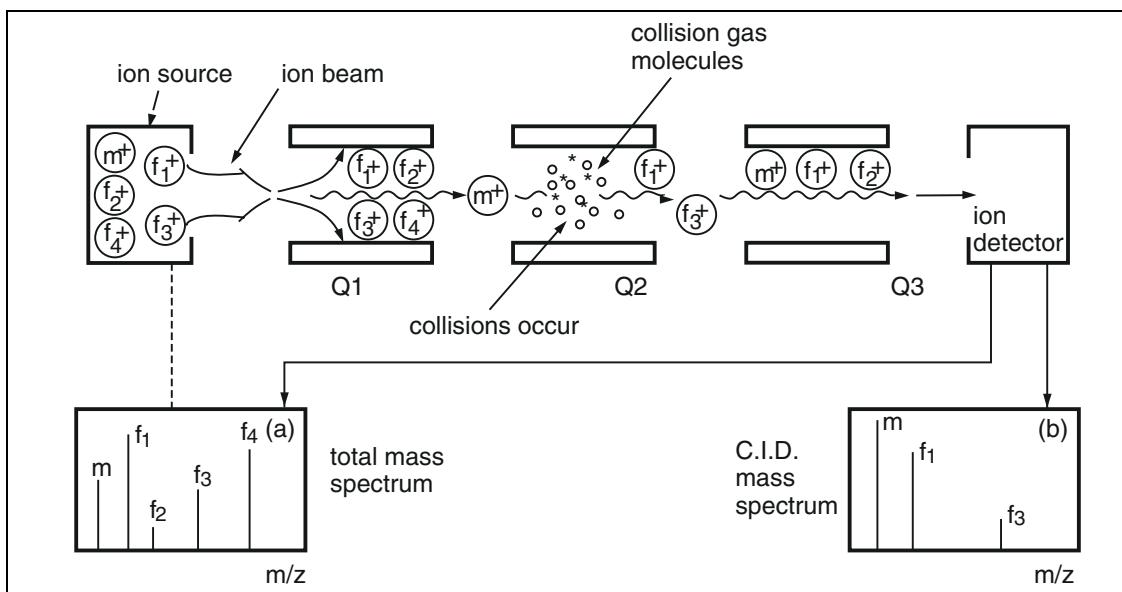


Figure 5 An example of linked scanning on a triple quadrupole instrument. A normal ion spectrum of all the ions in the ion source is obtained with no collision gas in Q2; all ions scanned by Q1 are simultaneously scanned by Q3 to give a total mass spectrum (a). With a collision gas in Q2 and Q1 set to pass only ions (m^+) in this example, fragment ions (f_1^+ , f_2^+) are produced and detected by Q3 to give the spectrum (b). This CID spectrum indicates that both f_1^+ and f_2^+ are formed directly from m^+ .

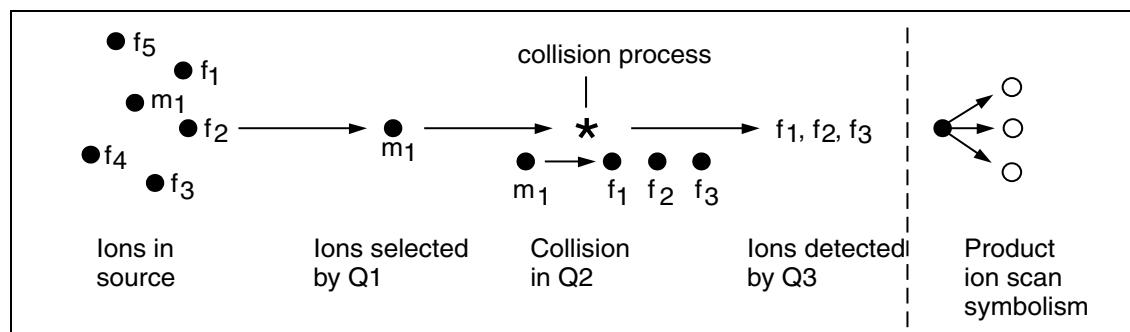


Figure 6 A product ion scan. Source ions (m_1^+ , f_1^+ f_5^+) are selected by setting Q1, in this case, to pass only m_1^+ . Collisional activation of these ions in Q2 induces dissociation to give, in this case, fragment ions (f_1^+ , f_2^+ , f_3^+) which are detected by scanning Q3. The symbolism for this process is shown.

By introducing a collision gas into Q2, collision induced dissociation (CID) can be used to cause more ions to fragment (Figure 5). For example, with a pressure of argon in Q2, normal ions (m_1^+) collide with gas molecules and dissociate to give m_2^+ ions. CID allows a bigger yield of fragments to be obtained than can be observed from 'natural' metastable ions formed without induced decomposition.

Linked Scanning with Triple Quadrupole Analysers

Linked scanning techniques by which fragmentation reactions can be examined are particularly easy to apply with QqQ instruments. The ease with which RF and DC voltages can be changed rapidly means that the scanning can be done very quickly. Three common, popular types of linked scan are briefly described here and serve to illustrate its principles (Figure 5).

(i) Product ion scans

Precursor ions are selected by Q1 and passed into the collision cell (Q2 or q2 of Figure 4). Here, collision with an inert gas (argon or helium) causes dissociation to occur and the resulting fragment (product) ions are detected by scanning Q3 (Figure 6).

(ii) Precursor ion scans

Quadrupole, Q3, is set to pass only the product ions under investigation. All the ions from the ion source are scanned by Q1 and passed successively into Q2 where collisional activation occurs. Those ions which fragment to give product ions of interest are revealed by the appearance of the product ions in Q3 (Figure 7).

(iii) Constant mass difference scans

For a process, $m_1^+ \rightarrow m_2^+ + n_0$, in which the difference in mass ($m_1 - m_2$) is a set value (Δm), the quadrupoles can be set to discriminate only that difference. Q1 scans the spectrum of ions from the source and Q3 scans the same masses but offset by Δm . For example with a set mass difference of 28 amu, if Q1 is scanning 80, 81, 82 then, concurrently, Q3 is scanning 52, 53, 54 The ions from Q1 are collisionally activated in Q2 so that, if they give ions in Q3 which are Δm mass units less, then the process, $m_1^+ \rightarrow m_2^+$ is established (Figure 8).

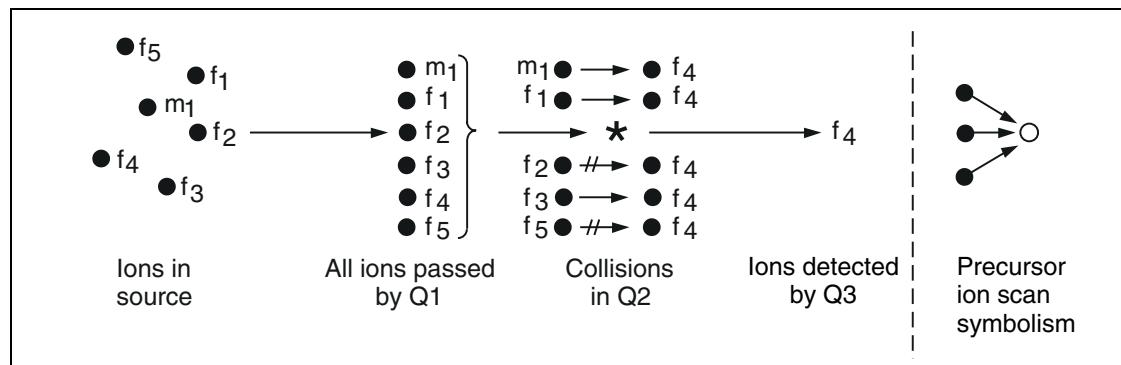


Figure 7 A precursor ion scan. Source ions ($m_1^+, f_1^+, \dots, f_5^+$) are all passed successively by Q1 into the collision cell, Q2, where a selected fragment (f_4) is produced and detected by Q3. Only the ions (m_1, f_1, f_3) give f_4 fragment ions in this example.

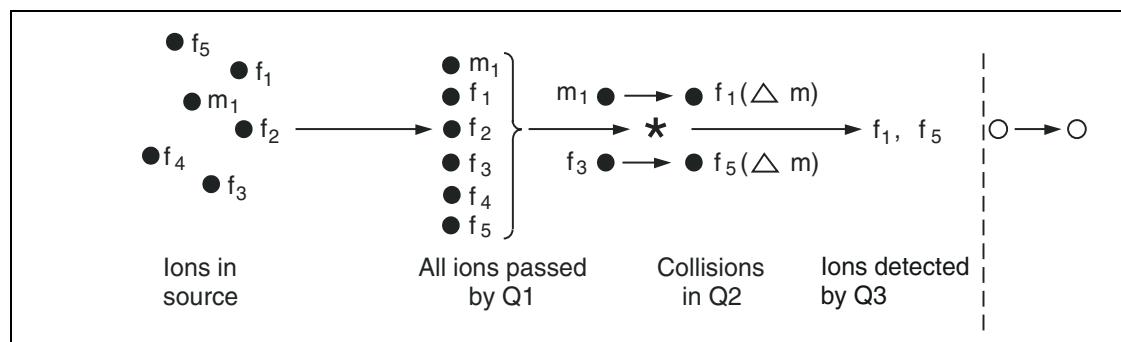


Figure 8 A constant mass difference scan. Source ions (m_1, f_1, \dots, f_5) are passed successively by Q1 into Q2, where collisionally induced dissociation occurs. Q3 is set to pass only those ions produced in Q2 which have a pre-determined mass difference (Δm) between the ions passed by Q1. In this example, they are $m_1 - f_1 (= \Delta m)$ and $f_3 - f_5 (= \Delta m)$ so that although all ions pass into Q2 only f_1, f_5 have a mass difference (Δm) equal to that selected for Q3. The symbolism is shown on the extreme right.

Use of Metastable Ion and CID Data

The importance of linked scanning of metastable ions or of ions formed by induced decomposition is discussed in the Guide 'Linked Scanning'. Briefly, linked scanning provides information on which ions give which others in a normal mass spectrum. With this sort of information, it becomes possible to examine a complex mixture of substances without prior separation of its components. It is possible to look highly specifically for trace components in mixtures under circumstances in which other techniques could not succeed. Finally, it is possible to gain information on the molecular structures of unknown compounds, as in peptide and protein sequencing (see Guide, 'Biotechnology').

Conclusion

Metastable and collisionally induced fragment ions can be detected efficiently by a triple quadrupole instrument. By linking the scanning regions of the first and third quadrupoles, various forms of linked scanning can be achieved.

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CHAPTER G1

GAS AND LIQUID CHROMATOGRAPHY

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Quick Guide

- Chromatography is a method for the separation of mixtures of substances into their individual components.
- Substances may be loosely categorised as volatile (including gaseous) and non-volatile. The terms are not exact.
- At normal sorts of pressures (around atmospheric) and up to about 250 °C (approaching the limit of thermal stability for most organic compounds), a volatile substance may be defined as one which can be vaporized by heat between ambient (10-30 °C) and 200-250 °C.
- By omission, non-volatile substances are all the remainder.
- Gas chromatography (GC) deals with substances which are volatile and can be vaporized into a gas stream.
- Liquid chromatography (LC) concerns mostly non-volatile substances dissolved in a liquid stream.
- It should be noted that LC can be used for volatile substances as well as involatile but GC can only handle volatile ones.
- Chromatography was originally a method for separating and displaying mixtures of coloured substances on a colourless column of solid material (chroma - colour; graph - writing).
- It was rapidly realised that this method could be used also to separate colourless substances but the name stuck and it is now simply a description of a process for separating any mixture, coloured or colourless, into its component parts.
- Rather like in a race, in chromatography, a mixture of substances (the runners) is placed at the beginning of a column (the track) and then made to move along the column (race) to the end, reached first by the faster ones and last by the slowest.
- Unlike an ordinary race, in chromatography the runners (substances) are forced along the track (column) by either a stream of gas (GC) or a stream of liquid (LC).

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- Finally some means must be provided for deciding when the separated substances are emerging from the end of the column, viz., a detector is needed.
- A mass spectrometer (MS) makes a very good, sensitive detector and can be coupled to GC to give the technique of GC/MS or to LC to give LC/MS.
- High pressure liquid chromatography (HPLC) is simply a variant on LC in which the moving liquid stream is forced along under high pressure in order to afford greater efficiency of separation.
- GC and LC (or HPLC) assume great importance as they are two of the most widely employed separation techniques in chemistry, biochemistry, pharmacology, and medical and environmental sciences.
- The coupled methods, GC/MS and LC/MS, form very powerful combinations for simultaneous separation and identification of components of mixtures. Hence, these techniques have been used in such widely disparate enterprises as looking for evidence of life forms on Mars and for testing racehorses or athletes for the presence of banned drugs.

Summary Mixtures of substances can be separated into their individual components by passage through special (chromatographic) columns in the gas phase or liquid phase.

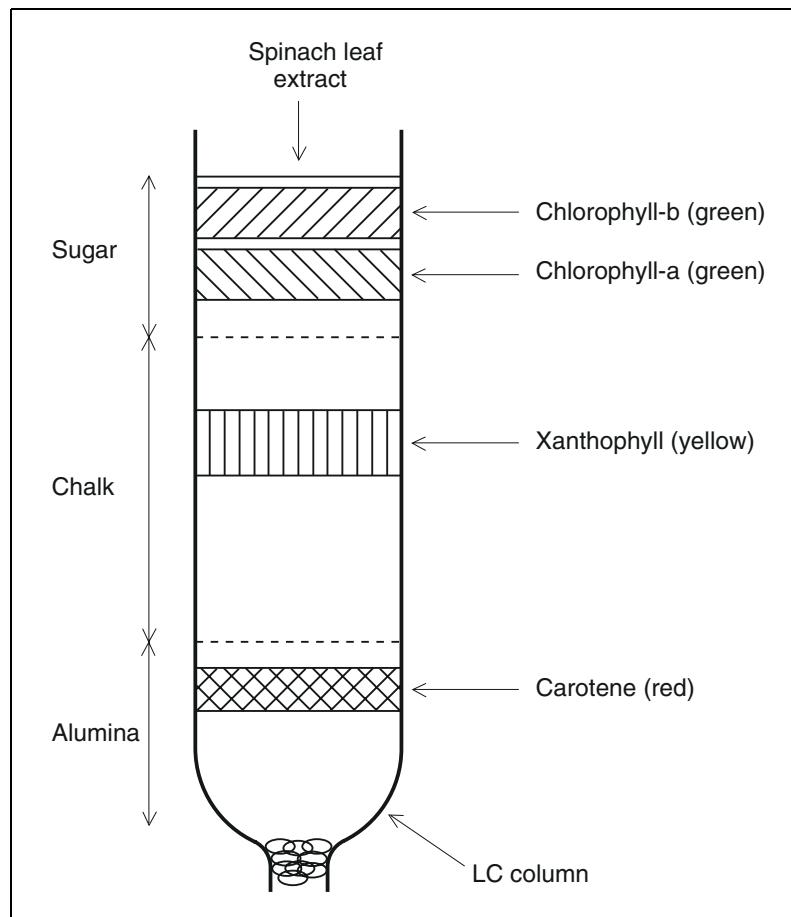


Figure I A chromatographic column filled in three sections with ground sugar, chalk and alumina. When a petroleum extract of spinach leaves is run onto the top of the column, the extract spreads down the column but not uniformly; bands of green chlorophylls stop near the top, yellow xanthophyll further down and red carotene near the bottom.

GAS AND LIQUID CHROMATOGRAPHY

Introduction

Chromatography is a general term used to describe the separation of mixtures into their individual components by causing them to pass through a column of a solid or liquid. The name derives from the first attempts at such a separation when the green and yellow colouring matters of spinach were revealed by putting an extract of it onto a column of a solid such as chalk or sugar held inside a glass tube. The components showed up as coloured bands observed at different positions along the column and hence the name (chroma - colour; graph - writing; Figure 1). Since then, chromatography has developed into a general technique for separation of any kind of mixture, coloured or non-coloured. It may be used for separating mixtures of gases, liquids or solids and has achieved enormous importance. Two particular forms of chromatography, gas and liquid, are used more than any other and have been enhanced even more by coupling them to mass spectrometers which act not only as a detection system (see below) but also provide valuable structural information about the separated components of mixtures. These coupled techniques of gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/mass spectrometry (LC/MS) are described in these 'Back-to-Basics' guides. Here, the principles of gas and liquid chromatography alone are discussed together with examples of their uses.

Principles of Gas and Liquid Chromatography

For chromatography to occur a mobile phase and a stationary phase are needed. The mobile phase is a gas in gas chromatography and is a liquid in liquid chromatography. In GC, the stationary phase is almost always a 'column' of liquid or gum or elastomer but not a solid whilst in LC the stationary phase is generally a 'column' of a porous solid. Strictly, the names should be gas liquid chromatography (GLC) and liquid solid chromatography (LSC), as will be found in the early literature, the more abbreviated names (GC, LC) coming into use through widespread use of the methods. It is the flow of the mobile phase through the stationary one that forces a mixture to pass through the chromatography column. During its passage through the column of stationary phase a mixture gradually separates into its individual components, some passing through the column of stationary phase faster than others and emerging (eluting) before them.

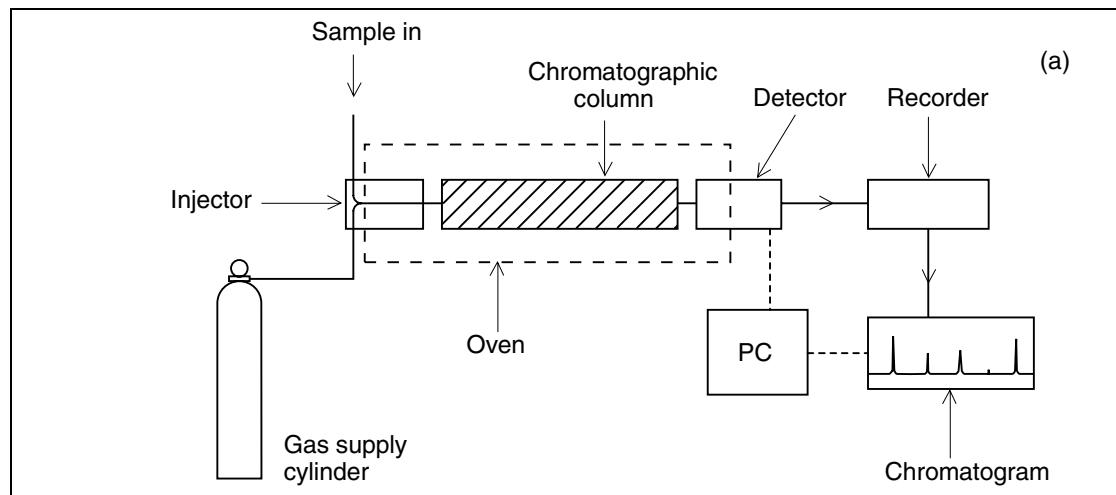


Figure 2a A schematic layout of a gas chromatograph. A sample is vaporized in the injector, mixed with nitrogen and is carried by the gas stream through a column where separation into components occurs. The emerging (eluting) components are detected and recorded to give a gas chromatogram. Alternatively, a personal computer (PC) can be used to acquire and display data. The injector, column and the lower part of the detector are placed in an oven.

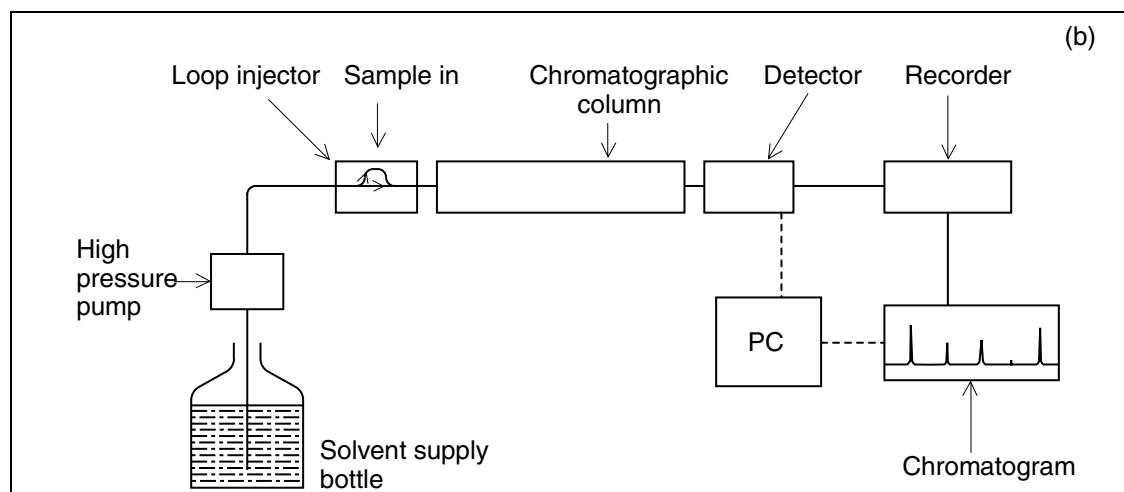


Figure 2b A schematic layout for a liquid chromatograph. Note the essential similarity to the gas chromatograph shown in 2a; however, in detail the injector, column and detector are quite different. No oven is needed.

Requirements for Chromatographic Apparatus

In principle, a chromatographic instrument is very simple and is little more than an advanced ‘plumbing’ system (Figure 2a, b). The mixture to be separated must be put onto the start (or top) of the column and so an injector is needed. The gas or liquid *mobile phase* flows from a suitable store (gas cylinder or bottle of solvent usually) past the injector and carries the injected mixture through the chromatographic column, which contains a stationary phase (a liquid for GC and a modified solid for LC). When the separated components emerge (elute) from the end (or bottom) of the column, they must be sensed by a detector. Finally, the detector must provide an electrical signal, directly or indirectly, to a read-out device or recorder. From injection of the mixture to recording the elution of individual components may take any time from a few seconds to an hour or more. The resulting chart from the recorder shows the amounts of separated components plotted against their times of elution and is called a chromatogram (Figure 3a, b). Many kinds of detector have been designed from the now generally used, cheap robust flame ionization (GC) or ultraviolet absorption type (LC) to the much more exciting and informative, if much more expensive, mass spectrometer.

The Chromatographic Process

The principles behind separation of mixtures by GC or LC are more or less the same but the experimental procedures are different. During chromatography, any one component of a mixture is partitioned between the mobile and stationary phases such that, at any one instant, some of the component is in one phase and the remainder is in the other. In this dynamic equilibrium system, progress of a component along the chromatographic column is effected by the flow of the mobile phase in a process of continual exchange between the stationary and mobile phases. The process can be likened simplistically to the movement of a bus along its route. For some of the time, the bus moves (molecules in the mobile phase) but, at other times, the bus is stopped (molecules in the stationary phase). The time taken by a bus to cover its route (retention time for molecules in chromatography) depends on the number and the length of time of the stops it makes and its speed between stops.

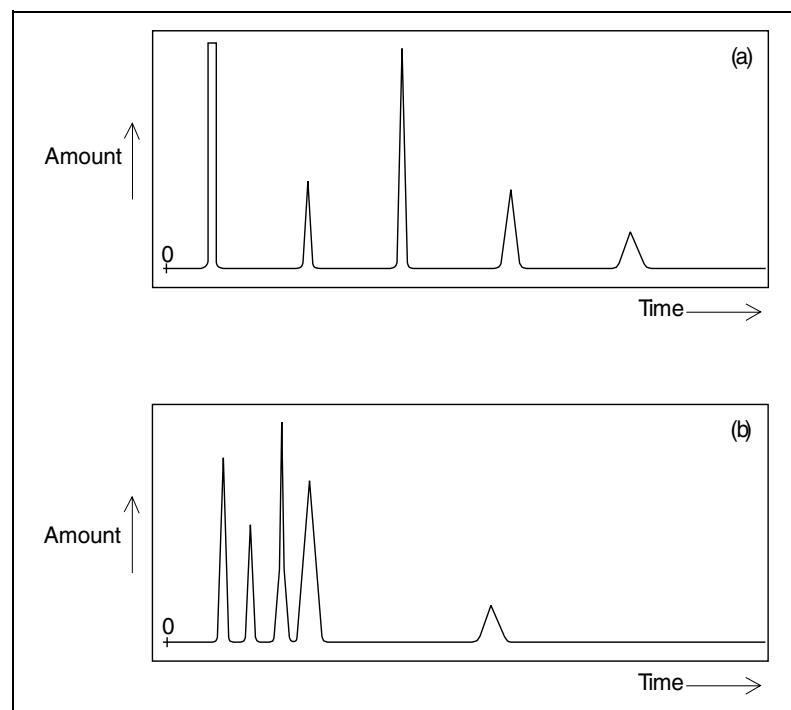


Figure 3 Typical (a) gas and, (b) liquid chromatograms. The charts show amounts (y axis) of substance emerging from a column versus time (x axis). The time taken (measured at the top of a peak) for a substance to elute is called a retention time.

Similarly, with molecules, their speed of movement through the chromatographic column depends on the time spent in the mobile phase compared with that in the stationary one and on the flow rate of the mobile phase.

(i) Gas Chromatographic Phases

For GC, the *mobile phase* is a gas (usually nitrogen or helium) and the movement of mixture components along the column will depend on their volatilities; the more volatile a component, the more time spent in the gas phase and the faster it will be swept along. This is a major but not the only factor controlling the rate of movement of a substance through a gas chromatographic column. The various components of a mixture will have different affinities for the *stationary phase*, a non-volatile liquid over which the mobile gas phase flows. Thus, for two components having the same volatilities but differing polarities, their rates of passage will be different. A polar component will tend to 'stick' on a polar stationary phase but not on a non-polar one whilst non-polar compounds will be held more strongly by non-polar stationary phases. The ability of a stationary phase to separate mixtures in GC depends on its chemical composition. Many types of stationary phase have been used, a few of which are listed in Table 1. An example of the change in gas chromatographic behaviour for two different columns of the same length is shown in Figure 4.

Because volatility is such an important factor in GC, the chromatographic column is contained in an oven, the temperature of which can be closely and reproducibly controlled. For very volatile compounds, the oven may be operated at only 30-40 °C but, for relatively involatile substances, temperatures of 200-250 °C may be needed. For GC analysis, the column may be operated at one fixed temperature (isothermally) or over a temperature range (temperature programming).

In general, the longer a chromatographic column the better will be separation of mixture components. In modern gas chromatography, columns are usually made from quartz and tend to be very long (coiled), often 10-50 m and narrow (0.1-1.0 mm, internal diameter) and hence their common name of capillary columns. The stationary phase is coated very thinly on the whole length of the inside wall of the capillary column. Typically, the mobile gas phase flows over the stationary phase in the column at a rate of about 1-2 ml/min.

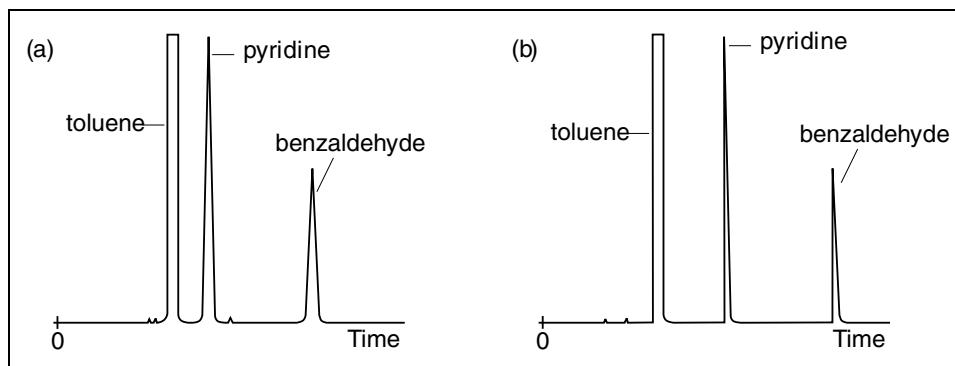


Figure 4 Two gas chromatograms showing the effect of polarity of the stationary phase on the separation efficiency for three substances of increasing polarity toluene, pyridine and benzaldehyde. (a) Separation on silicone SE-30, a relatively non-polar phase and, (b) on elastomer OV-351 a more polar one. Note the greatly changed absolute and relative retention times, the more polar pyridine and benzaldehyde being affected most by the move to a more polar stationary phase.

Table I Some Typical Stationary Phases for Use in GC

Name	Type	Approximate Polarity
Nujol	Mineral oil	Non-polar (11)*
Silicone SE-30	Elastomer	Non-polar (41)
Apiezon L	Grease	Non-polar (42)
Silicone OV-17	Elastomer	Intermediate (202)
Didecyl phthalate	Pure liquid	Intermediate (235)
Carbowax 20M	Polymer	Polar (510)
DEGS	Polymer	Very Polar (835)

* The numbers in parentheses (McReynolds Numbers) give an approximate indication of polarity on a scale of 0 (non-polar) to about 1000 (extremely polar).

(ii) Liquid Chromatographic Phases

For LC, temperature is not as important as in GC because volatility is not important and, usually, columns are metal and are operated at or near ambient temperatures so that the sort of temperature controlled oven used for GC is unnecessary. An LC *mobile phase* is a solvent such as water, methanol or acetonitrile and, if only a single solvent is used for analysis, the chromatography is said to be isocratic. Alternatively, mixtures of solvents can be employed. In fact, chromatography may start with one single or mixture of solvents and gradually change to a different mix of solvents as analysis proceeds (gradient elution).

The *stationary phase* in LC is a fine granular solid such as silica gel. It may be used as such (mainly for non-polar compounds) or the granules may be modified by a surface-bonded coating which changes (reverses) the polarity of the gel, a very small selection of stationary phases is listed in Table 2. These reversed phase columns are used for separation of polar substances. Although in LC the stationary phase is a solid, it is necessary to bear in mind that there may nevertheless be a thin film of liquid (e.g. water) held on its surface and this will modify the behaviour of sample components equilibrating between the mobile and stationary phases. A textbook on LC should be consulted for deeper discussion on such aspects.

In LC, because the mobile phase is a liquid and the stationary one is a granular solid, viscosity limitations rule out the simple use of the long capillary columns found in GC. Short columns of 10-25 cm and 2-4 mm internal diameter are more usual in LC. It is difficult to force the mobile solvent phase through such columns and high pressure pumps must be used to get a reasonable flow rate - hence the name, high pressure liquid chromatography (HPLC). More recently, very narrow LC columns have become available but these nano columns must be operated at very high pressure in order to force the mobile liquid phase along them.

Typically, a liquid phase flow of some 1-2 ml/min at a pressure of 20-200 bar (30-300 psi) is used. Very often the high pressure part of the terminology is omitted, as in LC/MS rather than HPLC/MS

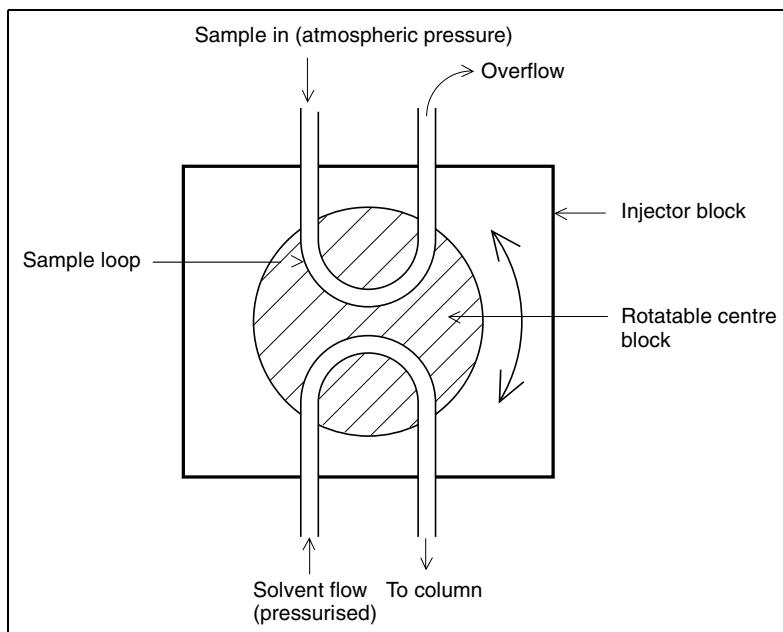


Figure 5 A typical loop injector showing the sampling position with pressurised solvent flowing through one loop onto the column and the sample solution placed in the other loop at atmospheric pressure. Rotation of the loop carrier through 180° puts the sample into the liquid flow at high pressure with only momentary change in pressure in the system.

Table 2 Some Solid Stationary Phases for Use in LC

Name	Polarity
Bondapak C18	Non-polar
Zorbax ODS	Non-polar
Carbowax 400	Intermediate
Micropak CN	Intermediate
Corasil II	Polar
Silica gel	Very Polar

Injectors

For GC, the injector is most frequently a small heated space attached to the start of the column. A sample of the mixture to be analysed is injected into this space by use of a syringe, which pierces a rubber septum. The injector needs to be hot enough to immediately vaporize the sample which is then swept onto the head of the column by the mobile gas phase. Generally, the injector is kept about 50 °C higher temperature than the column oven. Variants on this principle are in use, in particular the split/splitless injector; this can be used in one mode in which all of the injected sample goes onto the column (splitless) or in another mode (split) in which only part of the sample goes onto the column, the remainder being vented to atmosphere. For other less usual forms of injector, a specialist book on GC should be consulted.

For liquid chromatography, a sample of a solution of the mixture sample is injected through a loop injector. This allows a quantity of the solution to be placed in a small tubular loop at atmospheric pressure. By manipulating a valve, the high pressure flow of solvent to the column is diverted through the loop, carrying the sample with it (Figure 5).

Detectors

For GC/MS and LC/MS, specialized detectors and inlet systems are described in the relevant 'Back-to-Basics' guides.

The effluent from the end of a GC column is usually nitrogen or helium, which contain a very small proportion of organics as and when these emerge (elute) from the column. The most widely used detector is one in which the eluate is burned continuously in a flame after admixing it with hydrogen and air (flame ionization detector). Ions and electrons formed when emerging organics burn in the flame are monitored electronically, the resulting electric current being used to drive a chart recorder (Figure 6).

In LC, the most common means for monitoring the eluant is to pass it through a cell connected into an ultraviolet spectrometer. As substances elute from the column their ultraviolet absorption is measured and recorded. Alternatively, the refractive index of the eluant is monitored since this varies from the value for a pure solvent when it contains organics from the column.

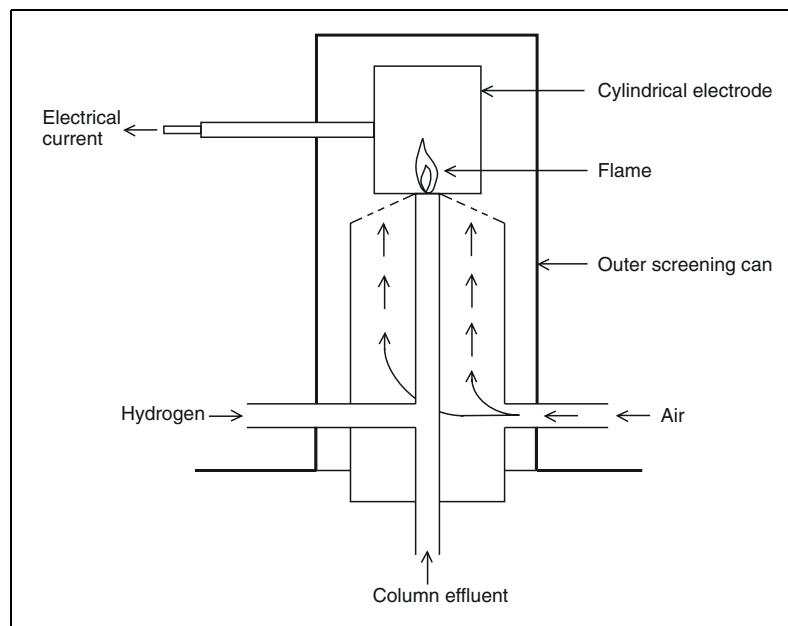


Figure 6 Schematic diagram of a flame ionization detector. Ions and electrons formed in the flame provide an electrically conducting path between the flame at earth potential and an insulated cylindrical metal electrode at high potential surrounding the flame; the flow of current is monitored, amplified and passed to the recording system.

Uses of GC and LC

As a 'rule-of-thumb' one can say that the efficiency of separation of mixtures and the simplicity of operating and maintaining apparatus are much greater for GC than for LC. Hence, other things being equal, GC is most often the technique of first choice and can be used with a very wide variety of compound types. However, for involatile or thermally labile substances like peptides, proteins, nucleotides, sugars, carbohydrates and many organometallics GC may be ruled out completely and LC comes into its own. Apart from availability of instrumentation, in choosing between GC and LC, it is necessary to consider the nature of the sample to be analysed. Whereas GC cannot be used with involatile or thermally labile compounds, LC can be used for almost all the types of substance routinely analysed by GC. The major reasons for LC not simply being used for all types of compound lie mostly in the far greater achievable resolution of mixtures into their components by GC, the generally greater sensitivity of GC detectors (nanogram amounts can be analysed easily) and the easier operation of GC instruments.

Conclusion

By passing mixtures through special columns (chromatography) in the gas phase (GC) or liquid phase (LC), they may be separated into their individual components and analysed qualitatively and/or quantitatively. The two types of chromatography have been directly coupled to mass spectrometers.

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Back to Basics Section G: Applications

CHAPTER G1.2

LC/MS

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Quick Guide

- This Guide is best read in conjunction with that entitled Gas Chromatography/Liquid Chromatography and any others concerning the operation of a mass spectrometer.
- Liquid Chromatography/Mass Spectrometry (LC/MS) is an analytical technique combining the advantages of an LC instrument with those of a mass spectrometer.
- LC, or sometimes HPLC (High Pressure Liquid Chromatography), is a means of separating components of mixtures by passing them in a solvent through a chromatographic column so that they emerge sequentially.
- With highly efficient chromatographic columns, very small amounts of complex mixtures can be separated in the liquid phase. Generally, the separated components cannot be positively identified by LC alone.
- MS is a means of examining a compound, in the gas phase, so that its structure or identity can be deduced from its mass spectrum. MS alone is not good for examining mixtures because the mass spectrum of a mixture is actually a complex of overlapping spectra from the individual components.
- Because an LC operates in the liquid phase but MS is a gas phase method, it is not a simple matter to connect the two. In order to allow separated components of a mixture to be passed sequentially from the LC into the MS an interface is needed. Having interfaced the two, LC/MS becomes a very powerful analytical technique.
- LC/MS allows more information to be gained than is obvious from the simple sum of the two separate instruments.
- LC can be combined with all kinds of mass spectrometer but, for practical reasons, usually only quadrupolar, magnetic/electric sector and TOF instruments are in vogue. A number of interfaces is in use: thermospray, plasmaspray, electrospray, dynamic FAB, particle beam and moving belt. The relevant 'Back-to-Basics' Guides should be consulted.
- A good LC/MS instrument routinely provides a means for obtaining the identities and amounts of mixture components rapidly and efficiently and it is not unusual to examine micrograms or less of material.

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- The above desirable properties of LC/MS make it popular for a diverse range of interests, including environmental, archaeological, medical, forensic and space sciences, chemistry, biochemistry and control boards for athletics and horse racing.

Summary By attaching an LC instrument to an MS through an interface, the resulting powerful combination of LC/MS can be used to analyse complex mixtures arising from a wide variety of sources both qualitatively and quantitatively.

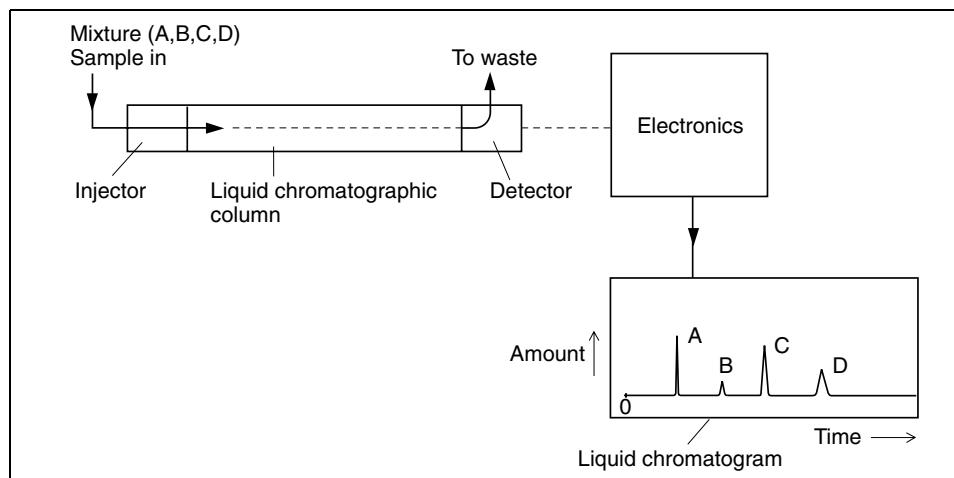


Figure 1 Schematic diagram showing injection of a mixture of four substances (A, B, C, D) onto an LC column, followed by their separation into individual components, their detection and the display (chromatogram) of the separated materials emerging at different times from the column.

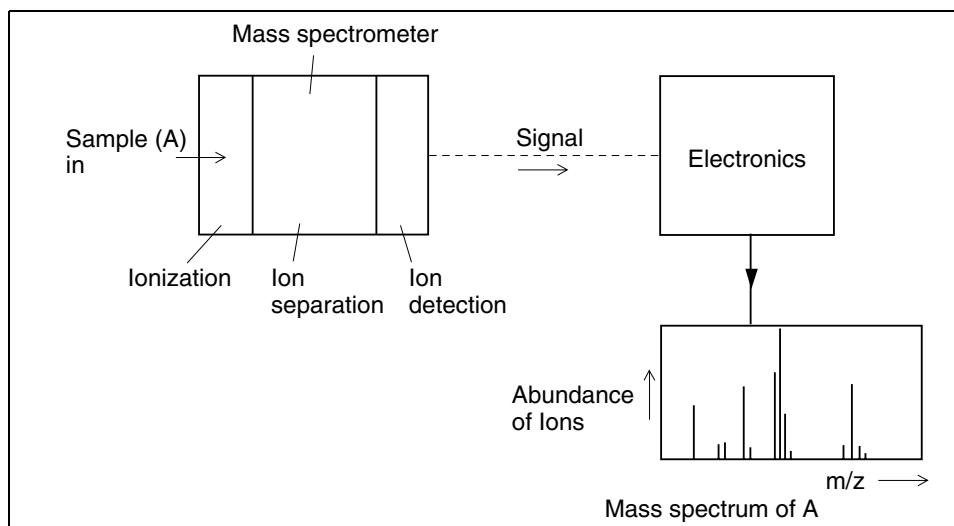


Figure 2 Schematic diagram of a mass spectrometer. After insertion of a sample (A), it is ionized, the ions are separated according to m/z value and, finally, the numbers of ions (abundances) at each m/z value are plotted against m/z to give the mass spectrum of A. By studying the mass spectrum, A can be identified.

LIQUID CHROMATOGRAPHY / MASS SPECTROMETRY

Introduction

As its name implies this important analytical technique is composed of two separate ones, liquid chromatography (LC) and mass spectrometry (MS), which have been combined. Historically, both individual techniques are quite old. LC developed as a means for separating involatile mixtures into their component substances and provided a big step forward in revealing their complexities and analysing them. The method is described fully in the relevant 'Back-to-Basics' Guide but here it might be summarized (Figure 1): by passing a mixture in a liquid stream (the mobile or liquid phase) through a long column packed with a stationary phase (particles of a special solid), the components of the mixture become separated and emerge (elute) one after another from the end of the column. In a simple LC instrument, the emerging components dissolved in the liquid mobile phase are measured by passing the liquid stream through either a UV or a refractive index detector. The detected components are recorded as 'peaks' on a chart (the liquid chromatogram), the area of a peak correlating with the amount of a component and the time taken to pass through the column (the retention time) giving some information on the possible identity of the component. The identification is seldom absolutely certain and is often either vague or not possible at all.

In complete contrast to an LC apparatus, a mass spectrometer is generally not useful for dealing with mixtures. If a single substance is put into a mass spectrometer, its mass spectrum can be obtained with a variety of ionization methods (Figure 2) for which the relevant 'Back-to-Basics' Guides should be consulted. Having obtained the spectrum it is then often possible to make a positive identification of the substance or to confirm its molecular structure. Clearly, if a mixture of substances is put into the MS the resulting mass spectrum will be a summation of the spectra of all the components (Figure 3). This spectrum will be extremely complex and it would be impossible to positively identify the various components (note that there are some MS instruments which can deal with mixtures but these are described in the 'MS/MS' and 'Linked Scanning' Guides). Thus, there is one instrument (LC) that is highly efficient for separating mixtures into their components but is not good on identification and another instrument (MS) which is efficient at identifying single substances but is not good with mixtures.

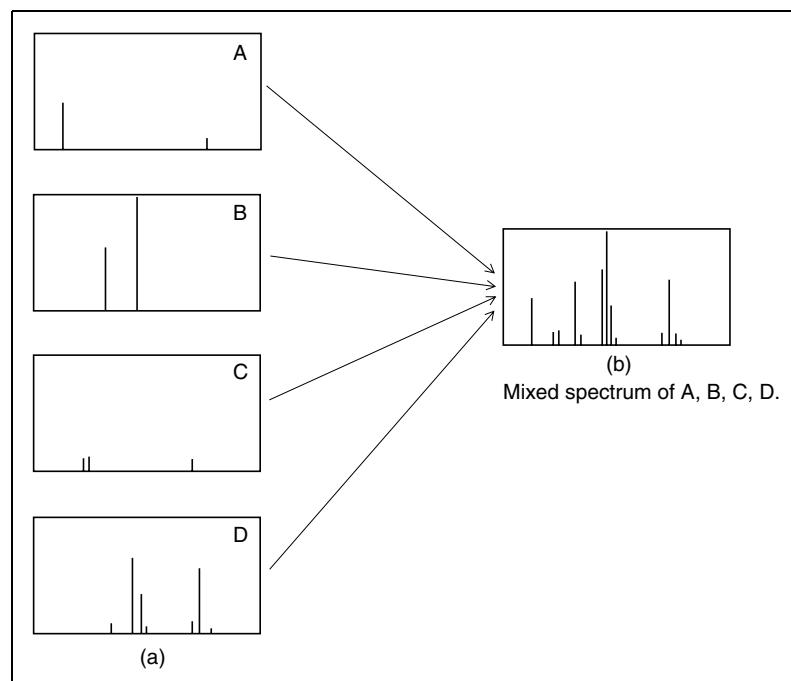


Figure 3 By way of illustration, very simple spectra for four substances (A, B, C, D) are shown, (a) separately and, (b) mixed in unequal proportions. The mixture spectrum is virtually impossible to decode if A, B,C, D are not known beforehand to be present.

It is not surprising to find early efforts being made to combine the two into one system (LC/MS) capable of separating, positively identifying and quantifying complex mixtures.

Combining LC and MS is much more difficult than was the case with GC and MS but modern LC/MS is now an established routine in many different kinds of laboratories. Further, the addition of LC to MS does not simply give a sum of the two alone; the information provided by combined LC/MS yields information that could not be extracted from the two isolated techniques, an aspect which is discussed below.

The Connection Between LC and MS

As described above, the mobile phase carrying mixture components along a liquid chromatographic column is a liquid, usually one of a range of solvents of which water, acetonitrile and methanol are common. This liquid flows from the end of the column at a rate of generally about 0.5 to 3.0 ml/min. In very narrow "nanocolumns" it is even less. This liquid flow must be transferred into the ion source of the mass spectrometer, which is under high vacuum. The large difference in pressure between the end of the chromatographic column and the inside of the ion source would cause enormous problems for the mass spectrometer vacuum system if there were no interface since a liquid stream of 0.5 to 3.0 ml/min. from the LC would almost immediately become a gas stream of hundreds of litres per minute. in the MS. Further, in this expansion, the mixture components would become diluted so that, even if the MS system could deal with the expanding solvent vapour, the sensitivity of detection of mixture components would be reduced catastrophically. Some means must be found for removing most of the liquid flowing from the column without losing the mixture components dissolved in it. Several methods (interfaces or separators) of overcoming this problem have been advanced, all with advantages and disadvantages that need to be considered.

In the earliest interface a continuous moving belt (loop) was used onto which the liquid emerging from the chromatographic column was placed as a succession of drops. As the belt moved along, the drops were heated at a relatively low temperature to evaporate the solvent and leave behind any mixture components. Finally, the "dried" components were carried into the ion source where they were heated strongly to volatilize them, after which they were ionized.

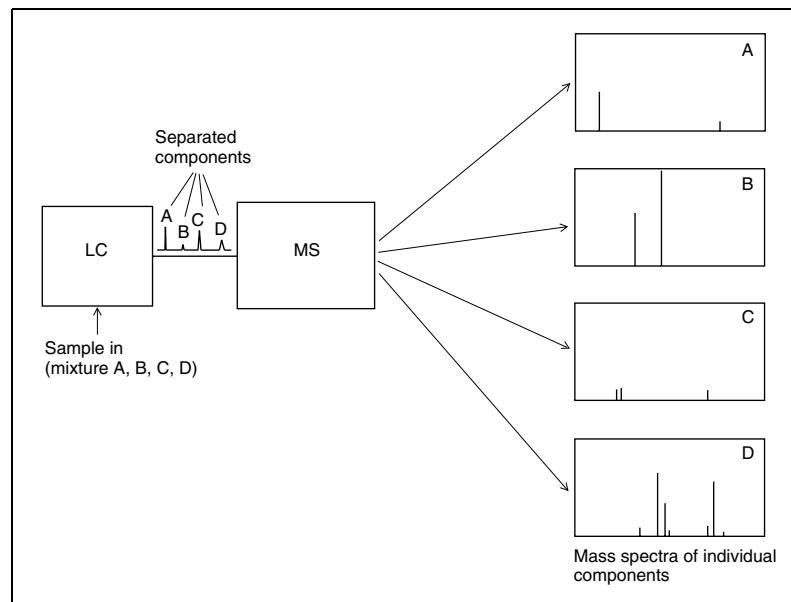


Figure 4 In an LC/MS combination, passage of the separated components (A, B, C, D) successively into the mass spectrometer yields their individual spectra.

This method is still in use but is not described in these 'Back-to-Basics' Guides because it has been superseded by more recent developments, such as particle beam and electrospray. These newer techniques have no moving parts, are quite robust and can handle a wide variety of compound types. They are fully described in these Guides (see: atmospheric pressure ionization, plasmaspray, thermo-spray, electrospray, dynamic FAB and particle beam [LincTM]).

It is worth noting that some of these methods are both an inlet system to the mass spectrometer *and* an ion source at the same time and are not used with conventional ion sources. Thus, with electrospray, the process of removing the liquid phase from the column eluant also produces ions of any emerging mixture components and these are passed straight to the mass spectrometer analyser; no separate ion source is needed. The particle beam method is different in that the liquid phase is removed and any residual mixture components are passed into a conventional ion source (often electron ionization).

Finally, it should be pointed out here that the ions produced by the combined inlet and ion sources such as electrospray, plasmaspray and dynamic FAB are normally molecular or 'quasimolecular' ions and there is little or none of the fragmentation so useful for structural work and for identifying compounds through a library search. Whilst production of only a single type of molecular ion may be useful for obtaining the relative molecular mass of a substance or for revealing the complexity of a mixture, it is often not useful when identification needs to be done, as with most general analyses. Therefore, the ions resulting from these combined inlet/ion source interfaces must be given extra energy to induce fragmentation (see: 'Metastable Ions' and 'Linked Scanning' Guides). The particle beam system does not suffer from this problem.

Recording Mass Spectra

As each mixture component elutes, the resulting ions are analysed by the mass spectrometer to give a mass spectrum (Figure 4). Separated mixture components elute in a short time interval, often only lasting a few seconds. Thus, the amount of any one component in the ion source is not constant as its mass spectrum is being obtained but, rather, it starts off as zero, rises to a maximum, and drops back to zero.

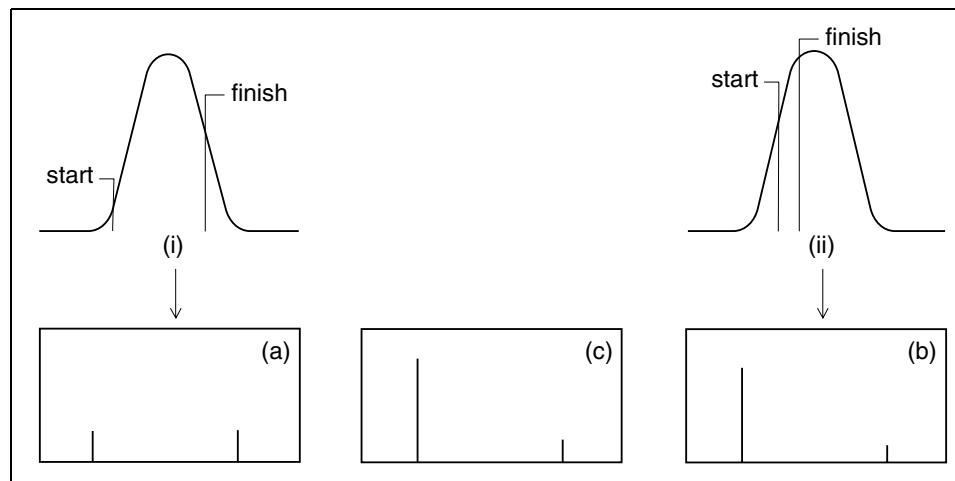


Figure 5 Slow scanning (i) of the mass spectrum over an LC peak for substance A gives spectrum (a) but rapid scanning (ii) gives spectrum (b) which is much closer to the true spectrum (c).

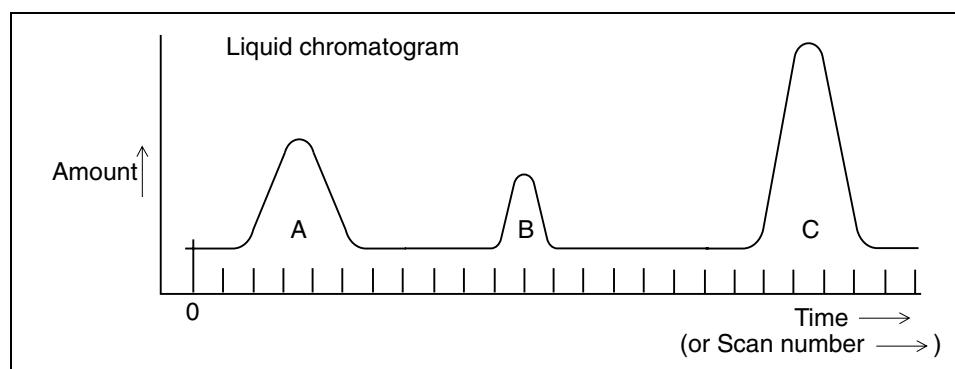


Figure 6 A typical liquid chromatogram showing three components (A, B, C) emerging at different times. Continuous scanning for mass spectra is started at zero and scans are repeated regularly (small tick marks). Thus, peak A is scanned five times during its passage through the ion source. Because scanning is regular, the 'time' axis can be replaced by a 'scan number' axis.

If this passage through the ion source is faster than the mass spectrometer can scan the spectrum, then a true spectrum will not be found because the start of the scan will 'see' less compound than at the middle of the scan and less again near the end; this changing concentration of eluting component results in a 'distorted' mass spectrum, which might well not be recognizable (Figure 5). The answer to this problem is to scan the spectrum so fast that, in effect, the concentration of the eluting component has scarcely changed during the time needed for obtaining a spectrum.

For a quadrupole mass spectrometer this high rate of scanning is not difficult because it requires only simple changes in some electrical voltages and these changes can be made electronically at very high speed. This is one reason for quadrupoles being popular in LC/MS combinations. In the early days of magnetic sector mass spectrometers the required scanning speed was just not possible because of serious hysteresis effects in the magnets. With modern magnet technology, scanning can be done at high speed with insignificant hysteresis and so magnetic sector instruments can compete with quadrupoles. Whilst ultimate scan speed for a magnetic instrument is not as good as the quadrupole's, the former does have an advantage in being able to provide greater mass resolution at higher mass.

As described above, the concentration of an eluting component in the ion source goes from zero to zero through a maximum. Where should the scan be taken? Usually, the greater the amount of a substance in an ion source the better the resulting mass spectrum (within reason!). This suggests that the best time for a scan will be near the maximum concentration (the 'top' of an LC peak) and that the instrument operator must watch the developing chromatogram continually, trying to judge when best to measure a spectrum. As a liquid chromatogram can routinely take 20 to 50 minutes to obtain, such a watching brief is labour intensive (and deadly dull!). The answer has been to simply set the mass spectrometer scanning continuously. Therefore, as the mixture is injected onto the chromatographic column, mass spectrometer scanning is instituted over a preset mass range (e.g., 50 - 500 mass units) at a preset interval (e.g., every 10 seconds). Thus, scan follows scan right through the chromatogram and, literally, hundreds of mass spectra may be recorded in a routine LC/MS experiment (Figure 6).

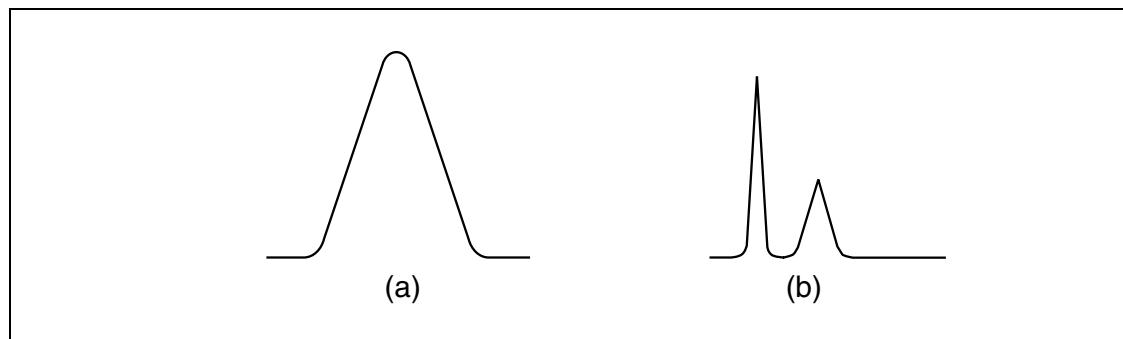


Figure 7 A 'single' peak from an ordinary liquid chromatogram (a) is revealed as two closely separated peaks by resolution enhancement (b).

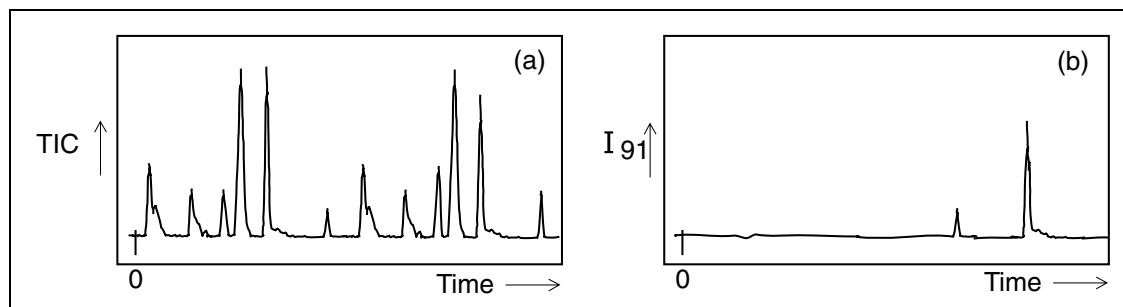


Figure 8 (a) A typical total ion current chromatogram, showing many components some of which have a part (R of characteristic mass, say 91). (b) A mass chromatogram based on m/z 91. The same data as used in (a) but now manipulated so that only ion currents corresponding to m/z 91 are plotted. Note the decrease in complexity, making the desired identification much easier.

Data flow from the mass spectrometer at such high speed and copious quantities that a microprocessor/computer is absolutely necessary to deal with it. At the end of an LC/MS 'run' all of the data are in storage, usually on a hard disc in the computer, in the form of a mass spectrum for every scan that was done. These spectra can then be manipulated. For example, the scan corresponding to the top of a chromatographic peak can be selected (scan 3 or 4 in Figure 6 for example) and the mass spectrum displayed or printed out. As a routine, the computer 'adds up' all the ion peaks in each mass spectrum (actually summing electrical currents) to give a total ion current for the mass spectrum. These total ion currents are plotted along an x axis (time for elution) and a y axis (amount of total ion current) to give a 'total ion current chromatogram' (TIC) showing the elution of all the components of a mixture. Storage of all these data has other major advantages.

Manipulation of Scan Data

Some ways in which data can be utilized are described briefly below.

(i) Having obtained a mass spectrum from an eluting component, the next step is to try to identify the component either through the skill of the mass spectroscopist or by resorting to a *library search*. If only molecular or quasimolecular ions have been produced, these will not be of much use for identification purposes. If the ions are given extra energy so that they fragment or if the particle beam or moving belt inlets are in place so that electron ionization can be used then the resulting mass spectra can be compared with those held in a large library of spectra from known compounds (e.g. the NIST library). There may be as many as 50-60,000 stored spectra in such a library, covering most of the known simple compounds likely to be met in analytical work. By special search routines under the control of the computer, this huge data base can be examined very quickly, comparing the mass spectrum of an eluted mixture component with each of the library spectra. The computer then provides a short list of the best matches between the library spectra and the measured one. Often, from the 'goodness' of match or 'fit' and its chromatographic retention time, the eluted component can be identified positively.

(ii) It happens not infrequently that a single peak from a liquid chromatogram may actually be due to not just one eluting substance but to two, three or more all eluting very close together.

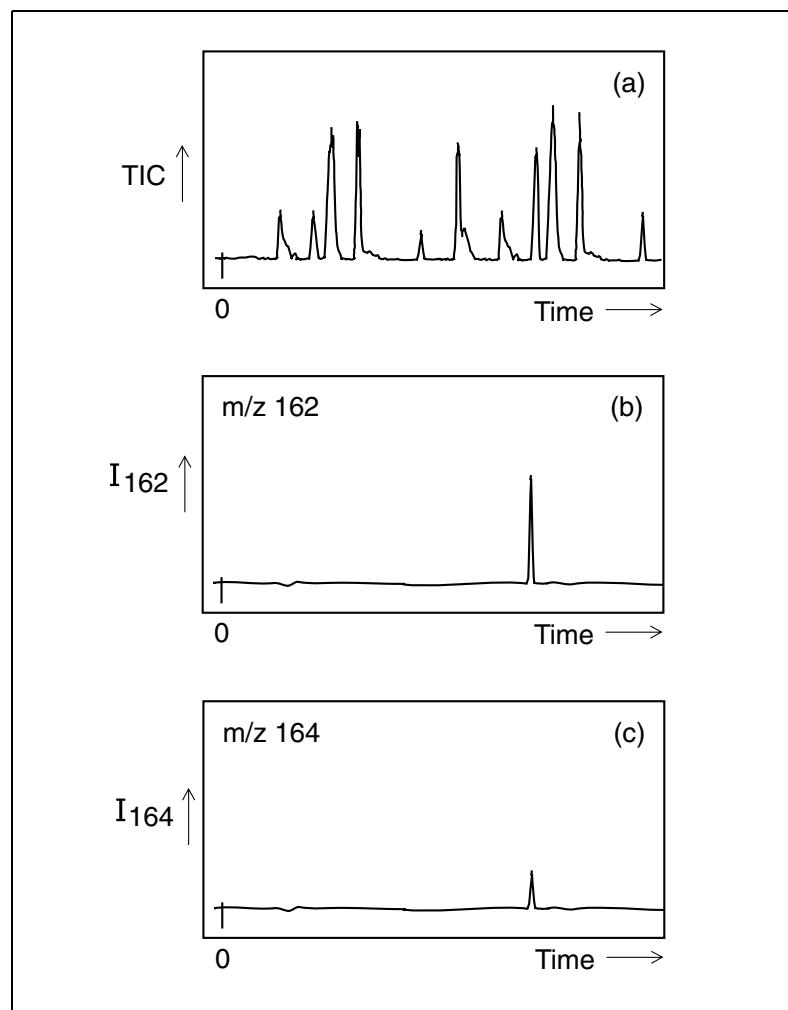


Figure 9 (a) A typical total ion current chromatogram showing many components of a mixture. The aim is to find if any chloronaphthalene ($C_{10}H_7Cl$; RMM = 162, 164) is present. To this end, the data (or a new chromatogram) are scanned at two specific positions, (b) at m/z 162 and, (c) at m/z 164. Note how m/z 162, 164 both reach a maximum at the same scan and have a ratio in peak heights of 3:1 ($^{35}\text{Cl}:\text{Cl} = 3:1$). Such an experiment in selected ion recording identifies the suspected component against a complex background and can be made very sensitive.

By examining the scans across the peak and using a simple mathematical process, the computer is able to reveal the existence of more than one eluting component in a 'single' peak and to print out the mass spectra of the discovered components. This is a form of 'resolution enhancement' viz., the LC/MS combination has been able to do what neither the LC nor the MS alone could do. In effect the mass spectrometer has improved (enhanced) the resolution of the liquid chromatograph (Figure 7).

(iii) There could be a series of compounds designated, RX, in which the part R was the same for all but X was different. In such a series the part R can give a 'characteristic ion' viz., the mass corresponding to R is the same for every member of the series. When one of such a series elutes from the chromatographic column its resulting mass spectrum will always contain one mass common to the whole series. Therefore, even if the series is mixed with other compounds, its members can be recognized from the characteristic ion. It is a simple process to get the computer to print out a chromatogram in which only those scans which contain the characteristic ion are used to draw the chromatogram. Basically, the output is blind to any component other than those containing an R group. Such a selected chromatogram is called a *mass chromatogram* and is useful for pinpointing where certain compounds elute without the need for examining all the spectra (Figure 8).

(iv) In a process somewhat similar to that described in item (iii) above, the stored data can be used to identify not just series of compounds but specific ones. For example, any compound containing a chlorine atom is obvious from its mass spectrum because natural chlorine occurs as two isotopes, ^{35}Cl and ^{37}Cl in a ratio of 3:1. Thus, its mass spectrum will have a two molecular ions separated by two mass units ($35+2=37$) in an abundance ratio of 3:1. It becomes a trivial exercise for the computer to print out only those scans in which two ions are found separated by two mass units in the abundance ratio of 3:1 (Figure 9). This selection of only certain ion masses is similar to 'selected ion recording' (SIR) or, sometimes, 'selected ion monitoring' (SIM).

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The ions selected for such recording can be one, two, three or more (*multiple ion recording*). In fact, through judicious choice of ions, the method can be so selective that a chosen component can be identified and quantified even though it could not even be observed in the original total ion current chromatogram. This very powerful technique is frequently used for examination of extremely complex mixtures in which it is desired to identify small amounts of a particular substance in a mass of other things, as with detection of banned drugs in the body fluids of athletes or racehorses or dioxins in industrial waste.

Conclusion

By connecting a liquid chromatograph to a suitable mass spectrometer through an interface and including a data system, the combined method of LC/MS (sometimes written HPLC/MS) can be used routinely for separation of complex mixtures into their individual components, for identification of the components and for estimation of their amounts. The technique is in very widespread use.

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Back to Basics Section G: Applications

CHAPTER G2

HR ACCURATE MASS - ELEMENTAL COMPOSITION

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Quick Guide

- Atoms have relative masses close to integer numbers: for example, hydrogen (H), is near one, helium (He) is near four and Nitrogen (N) is near fourteen.
- For molecules, the integer molecular mass is obtained by using the molecular formula and adding up the relevant individual masses. Ammonia (NH_3) has an integer mass of 17, made up of 1 \times 14 for N and 3 \times 1 for hydrogen.
- A mass spectrometer measures mass-to-charge ratio (m/z) and, often, the charge on the ion is unity so that $m/z = m/1 = m$. Thus, a mass spectrometer can be used to measure mass.
- For an ion of NH_3 , the measured integer mass would be 17, viz., $m/z = 17/1 = 17$, for $z = 1$.
- In theory, this process can be reversed in that any measured mass leads to an elemental composition. For example, a measured value of 17 would imply the composition, NH_3 .
- In practice, there are other elemental compositions which could add up to 17. For example, OH (oxygen = 16, hydrogen = 1), CDH_3 (carbon = 12, deuterium = 2).
- For larger masses, the possibilities increase enormously. At mass 100, there would be literally thousands of possible elemental compositions so that, although integer mass can be measured mass spectrometrically, attempts to obtain elemental compositions will not lead to a definite answer.
- In fact atomic masses are not integers. On the atomic scale, carbon is given a value of 12.0000. On this accurate mass scale, oxygen is 15.9949, nitrogen is 14.0031, hydrogen is 1.0078 and so on.
- The accurate mass for ammonia (NH_3) is:
$$14.0031 + 3 \times 1.0078 = 17.0265,$$
and OH is:
$$15.9949 + 1.0078 = 17.0027,$$
giving a mass difference of 0.0238 units between NH_3 and OH, which are potentially separable.
- A mass spectrometer which can measure mass correct to several decimal places rather than just integer mass can be used to measure such differences.

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- In the example given above, a measured mass of 17.0265 would indicate the definite composition, NH₃, and not the other possibility of OH.
- Even for large molecules the ability to measure accurate mass means that elemental compositions can be obtained from the accurately measured molecular mass.
- A simple mass spectrometer of low resolution (many quadrupoles, magnetic sectors, time-of-flight) cannot easily be used for accurate mass measurement and, usually, a double-focusing magnetic/electric sector or Fourier transform ion cyclotron resonance instrument is needed.
- Accurate mass measurement on a molecular ion of any substance gives directly the molecular formula; for fragment ions, similar measurement gives their elemental compositions.
- The double focusing mass spectrometer is arguably the finest instrument for obtaining molecular and elemental compositions.

Summary Accurate mass determination to several decimal places, using a double-focusing mass spectrometer, enables the determination of molecular formulae and elemental compositions of fragment ions.

Methane (CH_4)
$1 \times \text{carbon} + 4 \times \text{hydrogen} = (1 \times 12) + (4 \times 1)$
$= 16 (16.031)$
Water H_2O
$2 \times \text{hydrogen} + 1 \times \text{oxygen} = (2 \times 1) + (1 \times 16)$
$= 18 (18.01057)$
Ammonia (NH_3)
$1 \times \text{nitrogen} + 3 \times \text{hydrogen} = (1 \times 14) + (3 \times 1)$
$= 17 (17.0265)$
Ethanol ($\text{C}_2\text{H}_6\text{O}$)
$2 \times \text{carbon} + 6 \times \text{hydrogen} + 1 \times \text{oxygen}$
$= 2 \times 12 + 6 \times 1 + 1 \times 16$
$= 46 (46.0418)$
Glucose ($\text{C}_6\text{H}_{12}\text{O}$)
$6 \times \text{carbon} + 12 \times \text{hydrogen} + 1 \times \text{oxygen}$
$= 6 \times 12 + 12 \times 1 + 6 \times 16$
$= 192 (192.1634)$

Figure I Calculation of molecular mass from a molecular formula for several simple substances. Accurate masses are shown in parentheses.

Table I Relative integer and accurate atomic masses for some commoner elements.

Element	Symbol	Isotope	Integer Mass	Accurate Mass
Hydrogen	H	^1H	1	1.00783
(Deuterium)	(D)	^2H	2	2.01410
Carbon	C	^{12}C	12	12.00000
		^{13}C	13	13.00335
Nitrogen	N	^{14}N	14	14.00307
Oxygen	O	^{16}O	16	15.99491
Chlorine	Cl	^{35}Cl	35	34.96885
		^{37}Cl	37	36.96590
Silicon	Si	^{28}Si	28	27.97693

HIGH RESOLUTION ACCURATE MASS MEASUREMENT: ELEMENTAL COMPOSITIONS

Introduction

In many areas of science (chemistry, biochemistry, environmental, etc.), there is a need to determine the molecular formula of a substance. Thus, natural gas (methane) has a formula, CH₄, which indicates that each carbon atom (C) has four hydrogen atoms (H₄) attached; water has the formula, H₂O, indicating two hydrogens (H₂) attached to each oxygen. For more complex molecules, the formulae are correspondingly more complex, the natural product, adenosine, for example, has the formula, C₁₀H₁₃N₅O₄. In the past, the only way of obtaining such formulae was to break down a known weight of the molecule essentially into its constituent elements and weigh them, the resulting proportions giving the required formula. This was a painstaking, often not too accurate, slow process for complex molecules. The use of mass spectrometry for accurate mass measurement has transformed the situation such that, in modern science, molecular formulae for a vast range of substances are obtained using this technique.

Atomic and Molecular Mass: Fragment Ion Mass

The actual mass of an atom is very small indeed. For example, a hydrogen atom weighs something like 10⁻²⁴g. Instead of using such an absolute scale, a relative integer mass scale is easier to handle. On this relative scale, all atomic masses have values near to integers. Thus, the absolute masses for hydrogen (1x10⁻²⁴g) and deuterium (2x10⁻²⁴g) and relatively 1 and 2 respectively. Table 1 gives relative integer masses for some of the commoner elements.

Although this relative integer scale is acceptable in many circumstances where only approximate values are needed, for some applications, accurate relative masses are needed. By definition, on this accurate mass scale, carbon (¹²C) is given the value of exactly twelve, i.e., 12.00000. Table 1 indicates the accurate masses for some elements compared with their integer values.

Atoms combine in definite proportions to give molecules. For example, natural gas is mostly composed of methane, a substance in which four hydrogen atoms (H) are combined with one carbon (C); the molecular formula is written as CH₄. Similarly, water, ammonia, ethanol and glucose have the respective molecular formulae: H₂O, NH₃, C₂H₆O and C₆H₁₂O₆. For each of these substances a molecular mass can be computed, as detailed in Figure 1. Since these masses are calculated from *relative* atomic masses, they should be referred to strictly as relative molecular mass (RMM).

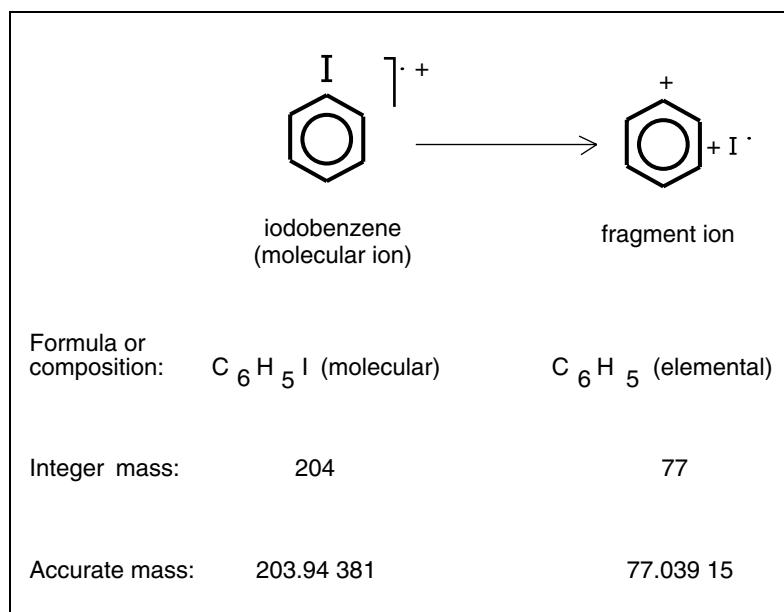


Figure 2 Simple fragmentation of the molecular ion of iodobenzene gives a fragment ion, $C_6H_5^+$. The difference in measured masses between the molecular and fragment ions gives the mass of the ejected neutral iodine atom.

Recent publications use RMM but older publications used molecular weight (MW or M.Wt). Values for the accurate relative molecular masses are also given in Figure 1.

In a mass spectrum, removal of an electron from a molecule (M) gives a molecular ion ($M^{•+}$; Equation 1).

The mass of an electron is very small compared with the mass of even the lightest element and, for all practical purposes, the mass of $M^{•+}$ is the same as that of M. Therefore mass measurement of a molecular ion gives the original relative molecular mass of the molecule.



Decomposition of ions gives fragment ions which can also be mass measured. Figure 2 illustrates a simple example in which the molecular ion of iodobenzene cleaves to give a fragment ion, $C_6H_5^+$, and an iodine atom, I. The molecular mass at 203.94381 corresponds to the molecular formula (or elemental composition) of C_6H_5I whilst the fragment ion mass at 77.03915 corresponds to the elemental composition, C_6H_5 ; the difference in mass equates to the mass of the ejected neutral iodine atom.

A suitable mass spectrometer can be set to measure relative atomic, molecular or fragment ion mass.

The Value of Accurate Mass Measurement

A mass spectrometer can measure integer relative mass with high accuracy but the result is not nearly so informative as would be measurement of accurate relative mass. An example illustrates the reason.

The three substances carbon monoxide (CO), ethene (C_2H_4) and nitrogen (N_2) each have an integer molecular mass of 28 (Figure 3). Their occurrence in a mass spectrometer would give molecular ions at integer masses of 28 and they could not be distinguished one from another. However, their accurate masses at 27.99491, 28.03132 and 28.00614 respectively are different and a mass spectrometer capable of accurate mass measurement would be able to distinguish them even if all three occurred in the same sample.

Thus, accurate mass measurement is useful for confirming the molecular composition of a tentatively identified material.

Chemical name	Formula	Integer Mass	Accurate Mass
Carbon monoxide	CO	28	27.99491
Ethene	C ₂ H ₄	28	28.03132
Nitrogen	N ₂	28	28.00614

Figure 3 Integer and accurate masses for three different gases, each having the same integer relative molecular mass (RMM = 28).

Table 2 A Listing of elemental compositions versus accurate mass at nominal integer mass of 58.

Integer Mass = 58				Accurate Mass
*C	H	N	O	
1	-	1	2	57.992902
1	2	2	1	58.016711
1	4	3	-	58.040520
2	2	-	2	58.005478
2	4	1	1	58.053096
2	6	2	-	58.053096
3	6	-	1	58.041862
3	8	1	-	58.065671
4	10	-	-	58.078247

* Compositions are read from left to right. Thus the fifth entry would be C₂H₄NO, of accurate mass 58.053096.

There is a more important use. Suppose a mass spectrometer has accurately measured the molecular mass of an *unknown* substance as 58.04189. Reference to tables of molecular mass versus elemental composition will reveal that the molecular formula is C₃H₆O (see Table 2). The molecular formula for an *unknown* substance can be determined and this helps enormously to identify it.

Finally, accurate mass measurement can be used to help unravel fragmentation mechanisms. A very simple example of this was given in Figure 2. If it is supposed that accurate mass measurements were made on the two ions at 203.94381 and 77.03915, then their difference in mass (126.90466) corresponds exactly to the atomic mass of iodine, showing that this atom must have been eliminated in the fragmentation reaction.

Resolution of Mass Spectrometers

The ability of a mass spectrometer to separate two mass (M₁, M₂) is termed *resolution* (R). The commonest definition of R is given by equation (2), in which $\Delta M = M_1 - M_2$ and $M = M_1 \approx M_2$. Thus if a mass spectrometer can separate two masses (100, 101) then $\Delta M = 1$, $M = 100$ and $R = 100$. For conventional accurate mass measurement, R needs to be as large as possible, typically having a value of 20,000. At this sort of resolution, a mass of 100.000 can be separated from a second mass at 100.0050 ($\Delta M = M/R = 100/20,000 = 0.005$)

$$R = M/\Delta M \quad (2)$$

To use the formula in equation (2), it is necessary to define at what stage the two peaks representing the two masses are actually separate (Figure 4); the depth of the 'valley' between the two peaks is used for this purpose, with 5, 10 and 50% valley definitions being in use. A 5% valley definition is a much stricter criterion of separation efficiency than is the 50% definition.

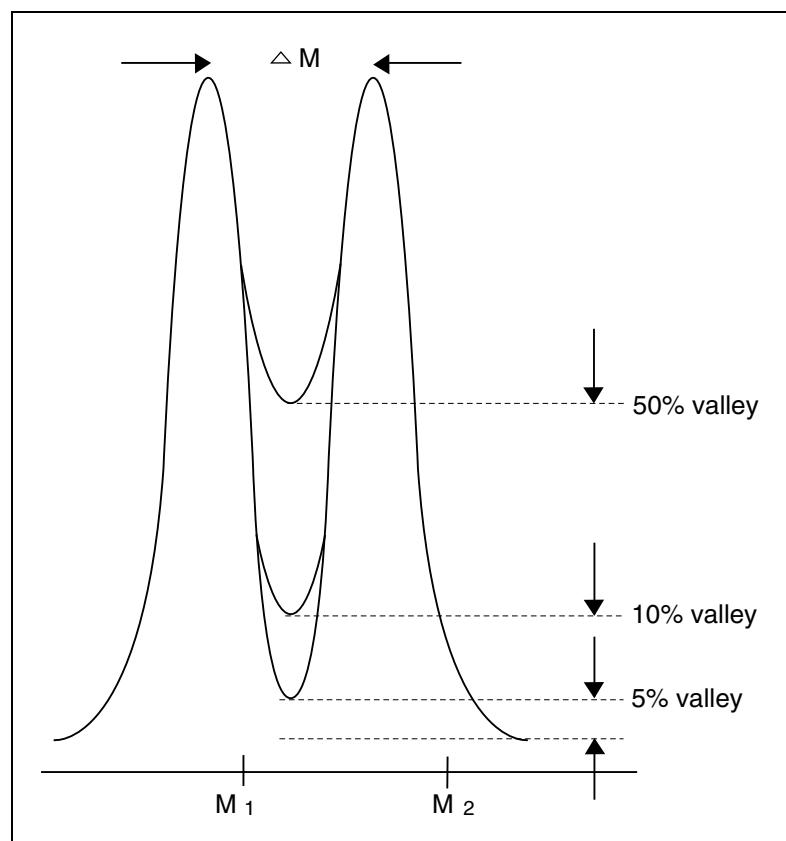


Figure 4 The separation (ΔM) of two peaks representing masses (M_1 , M_2) can be defined as having a 5 or 10 or 50% 'valley', viz., the depth of the valley is 5, 10 or 50% of peak height. The 5% definition is a more severe test of instrument performance.

Measurement of Accurate Mass

(a) By automated methods

All routine mass spectrometers measure accurate mass by reference to standard substances. High resolution accurate mass measurement requires that a *standard* substance and the sample under investigation be in the mass spectrometer at the same time. Ions from the standard have known mass and ions from the unknown (sample) are mass measured by interpolation between successive masses due to the standard (Figure 5). High resolution is needed so as to ensure that there will be mass separation between ions due to the standard and those due to the sample. For this reason, the standard substances are usually perfluorinated hydrocarbons because ions from these substances have masses somewhat less than an integer value whereas most other (organic) compounds have masses somewhat greater than an integer value. For example, at integer mass 124, the fluorocarbon, C_4F_4 , has an accurate mass of 123.9936 but a hydrocarbon, C_9H_{16} , has an accurate mass of 124.12528. A mass spectrometer resolution of 4000 is sufficient to separate these masses. The whole operation is usually automated by a data system but it can be done manually by a system of *peak matching*.

(b) By peak matching methods

In the example of Figure 5, for a magnetic sector instrument, two masses (M_I , M_u) will follow different trajectories in the mass spectrometer, defined by radii of curvature (r_I , r_u ; see Back-to-Basics on 'Ion Optics'). If the magnetic field strength is B and the ions were accelerated from the ion source through an electric potential of V volts then equations (3) follow. If now the voltage (V) is changed to V^* so that $r_I = r_u$, then equations (4) show that the unknown mass (M_u) can be calculated if mass (M_I) from the standard is known and the ratio of V^*/V is measured.

$$M_I/z = B^2 r_I^2 / 2V; \quad M_u /z = B^2 r_u^2 / 2V \quad (3)$$

Therefore, for accurate mass measurement, a standard mass peak (M_I) is selected, and the accelerating voltage (V) is changed until the sample ion peak (M_u) exactly coincides with the position of M_I ; this is peak matching and the ratio between the original and new voltages (V/V^*) multiplied by mass (M_I) gives the unknown mass, M_u .

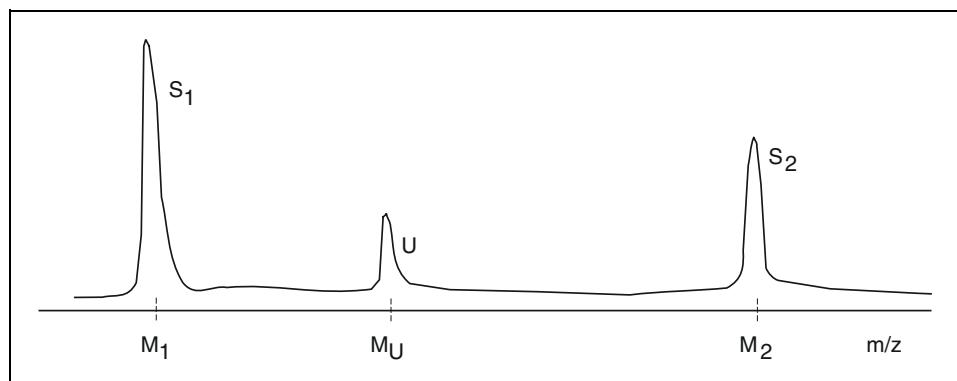


Figure 5 Part of a high resolution mass spectrum showing two peaks (S_1, S_2) due to ions of mass (M_1, M_2) from a standard substance and one peak (U) due to an ion of unknown mass (M_u) from a sample substance. The difference between M_1 and M_2 is accurately known and therefore the mass, M_u can be obtained by interpolation (the separation of M_u from M_1 or M_2). If the separation of M_1, M_2 is time (T) and that of M_1, M_u is time (t) then for a linear mass scale $M_u = [t(M_2 - M_1)/T] + M_1$.

For greatest accuracy, the standard mass (M_1) should be as close as possible to the unknown (M_u).

$$M_1/z = B_2 r_1^2 / 2\text{colour} \quad M_u/z = B_2 r_1^2 / 2V^*$$

$$\therefore M_u = M_1(V/V^*) \quad (4)$$

Peak matching can be done on quadrupole and magnetic sector mass spectrometers but only the latter, particularly as double-focusing instruments, have sufficiently high resolution for the technique to be useful at high mass.

(c) Other methods

Other techniques for mass measurement can or have been used but are not so popular as those outlined above. These other methods include measurement on a standard to 'calibrate' the instrument and then withdrawing the standard, letting in the unknown and comparing the new spectrum with that of the standard; it is assumed that there are no instrumental variations during this change-over. Generally, this technique is not so reliable as when the standard and unknown are in the instrument together. Fourier transform techniques are used with ion cyclotron mass spectrometers and give excellent mass accuracy at lower mass but not at higher.

Conclusion

By high resolution mass spectrometry, ions of known mass from a standard substance can be separated from ions of unknown mass derived from a sample substance. By measuring the unknown mass relative to the known ones through interpolation or peak matching, the unknown can be measured. An accurate mass can be used to obtain an elemental composition for an ion. If the latter is the molecular ion, the composition is the molecular formula.

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Back to Basics Section H: Miscellaneous

CHAPTER H3

AN INTRODUCTION TO BIOTECHNOLOGY

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Quick Guide

- Chromosomes are extremely complex chemicals which are assembled from relatively simple repeating units and contain all the chemical information needed to reproduce animate species. Each living organism has its own complete set of chromosomes, called the genome.
- Genes are segments of chromosomes. Some of the genes are coded to give each animate species its characteristics (e.g. colour and number of eyes, type of hair, muscle) and others are coded to produce the chemicals required for the organism to live (metabolism).
- Genes are constructed from sets of deoxyribonucleic acids (DNA) which, in turn, consist of chains of nucleotides. These chains occur in matched pairs, twisted around each other (the double helix).
- Any one nucleotide, the basic building block of a nucleic acid, is derived from a molecule of phosphoric acid, a molecule of a sugar (either deoxyribose or ribose) and a molecule of one of five nitrogen compounds (bases) - cytosine (C), thymine (T), adenine (A), guanine (G), uracil (U).
- A chain of nucleotides containing only deoxyribose as the sugar is a DNA. Similarly, RNA possesses chains nucleotides having only ribose as the sugar and is therefore a ribonucleic acid.
- The information needed to reproduce and support an animate species is given by the order in which the nitrogen bases occur along the DNA or RNA chains (-C-T-T-A-G-, for example). A sequence of three such bases (a codon) provides the fundamental unit of information.
- α -Amino acids are the molecular building blocks of peptides and proteins. About 20 amino acids are known, each corresponding to one or more codons.
- Peptides and proteins are formed by linking successive amino acids into chains or rings. The order (sequence) and types of amino acids (read from the sequence of codons) determine the chemical and physical properties of peptides and proteins (and enzymes).
- An enzyme is a special protein which acts as a catalyst for biochemical reactions (metabolism).

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- The genome, through its constituent DNAs, provides all of the codes needed for building a wide range of peptides, proteins and enzymes which in turn utilise raw materials (food) to form an animate body and keep it going. These multiple reactions work together as a unit within a water filled cell.
- Simple life-forms, such as bacteria, consist of single cells whereas, at the other extreme, complex life-forms such as animals, contain many types of cell, each having a specific function (cells in eyes, limbs, stomach and so on).
- Changes in genes (mutation) and therefore in genetic information, can occur naturally (mistakes in the billions of metabolic reactions) or accidentally through damage (cosmic particles, radioactivity, smoking and so on) or they can be effected intentionally and specifically by genetic engineering.
- Genetic engineering uses special chemicals (restriction enzymes) to snip DNA in specific places and others (ligases) to stitch cut ends. By this recombinant technology, gene DNA can be cut and a new piece inserted; the new segment may be natural (from another DNA) or synthetic (made automatically by a gene machine which makes lengths of DNA from nucleotides).
- Gene cloning is a method, using recombinant technology, for inserting a gene into a vector DNA (plasmid) obtained from a bacterium. The modified vector is put back into the bacterium which then reproduces endlessly (clones) the new gene as well as the others in the vector.
- A virus is a species containing DNA and RNA which can reproduce itself but, to do this, needs to hijack the metabolism (cells) of a host organism since it has no information itself with which to build cells.

Summary Life-forms are based on coded chemicals which, in the right environment, can reproduce themselves and make other chemicals needed to break down and utilise food. Within an organism, these biochemical reactions constitute normal metabolism. Biotechnology is the manipulation of these biochemical reactions at either the cellular or molecular level.

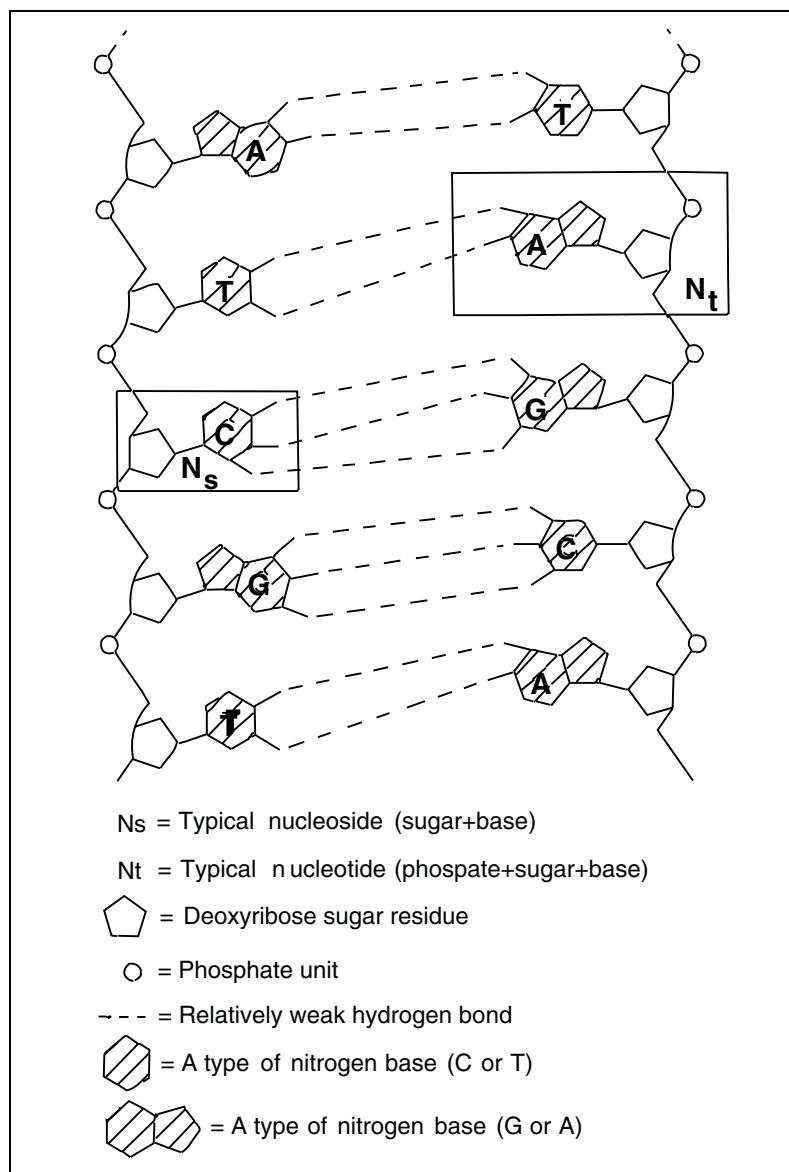


Figure I Schematic diagram of deoxyribonucleic acid (DNA) showing the pairing of nitrogen bases (A-T;C-G) between parts of two strands of DNA, the backbone of each strand being composed of sugar and phosphate units linked alternately.

AN INTRODUCTION TO BIOTECHNOLOGY

Genetics

Chromosomes,
DNA and Protein
Synthesis

All living matter, including humans, animals and plants, is made up of cells, which are small bags of saline water and a rich variety of chemicals. The cells are fundamental units of life. Cells must be able to make peptides, proteins and enzymes which are required to build an organism and to utilise raw material (food) to keep the organism alive. However, the most important property of all living cells is their ability to produce replicas of themselves without any external instructions. The study of the information content of cells, or heredity, is called genetics and the genetic information of a cell determines the distinguishing features of the organism.

The nucleus of the cell contains chromosomes, which appear in pairs. When cells divide, each new cell receives a complete set of chromosomes by uncoupling of the pairs. Chromosomes contain both proteins and deoxyribonucleic acids (DNA), the latter being the genetic material. DNAs are long, linear molecules, each having a backbone of alternating sugar (deoxyribose) and phosphate units; in addition each sugar is also attached to one of four possible bases: adenine (A), thymine (T), guanine (G) and cytosine (C) to form nucleosides and nucleotides (Figure 1). Two strands of DNA run in parallel but opposite directions in a helical configuration with relatively weak hydrogen bonds between the two strands.

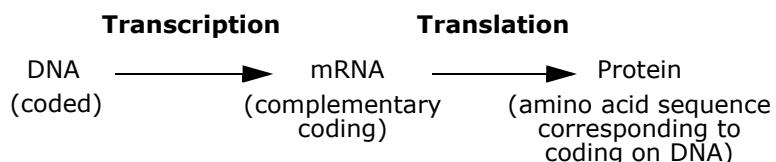
As shown in Figure 1, the bases appear in complementary pairs, A with T and G with C; in this particular example, the sequence for one strand of DNA is A-T-C-G-T- whilst the other strand is -T-A-G-C-A-. The sequences of the bases attached to the sugar-phosphate backbone direct the production of proteins from amino acids. Along each strand, groups of three bases, called codons correspond to individual amino acids.

For example, in Figure 1, the triplet CGT, acting as a codon, would correspond to the amino acid, serine. One codon, TAC, indicates where synthesis should begin in the DNA strand and others, such as ATT, indicate where synthesis should stop.

When the cell requires instructions for protein production, part of the code on DNA, starting at an initiator and ending at a stop codon, is converted into a more mobile form, by transferring the DNA code into a matching RNA code on a messenger ribonucleic acid (mRNA), a process known as transcription.

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The decoding, or translation, of mRNA then takes place by special transfer ribonucleic acids (tRNA) which recognise individual codons as amino acids. The sequence of amino acids is assembled into a protein (see 'Proteins' section) In summary, the codes on DNA are used to synthesize proteins:



This whole process can be likened to the translation of one language into another by means of two interpreters, one of whom (mRNA) writes down the words of the first language (DNA) and the second (tRNA) translates the words into the second language (protein).

Genetic Engineering and Gene Cloning

The term *genetic engineering* is applied to methods of altering an organism's characteristics by direct manipulation of its constituent DNA. For example, *genetic recombination* is the process in which a new DNA molecule is formed by chemically cutting and joining DNA strands using special enzymes. *Transposition* refers to the transfer of a gene from one chromosome to another or from one site to a different one on the same chromosome, using this *recombinant technology*. Much recombinant work is done with natural gene (DNA) material but synthetic DNA can be used. Synthesis of specified lengths of DNA (or RNA) from constituent nucleotides can be carried out using special automatic apparatus - a *gene machine*. These 'artificial' lengths of DNA, sometimes called oligonucleotides, can be spliced into chromosomal DNA by recombinant technology just as natural DNA can.

Gene cloning, or exact copying, is achieved by the use of microorganisms. Fragments of DNA containing just one or a few genes can be taken from any source and placed in one of the nucleic acids of a microorganism such as E.coli which then treats the new DNA as if it were its own and produces millions of exact copies (clones).

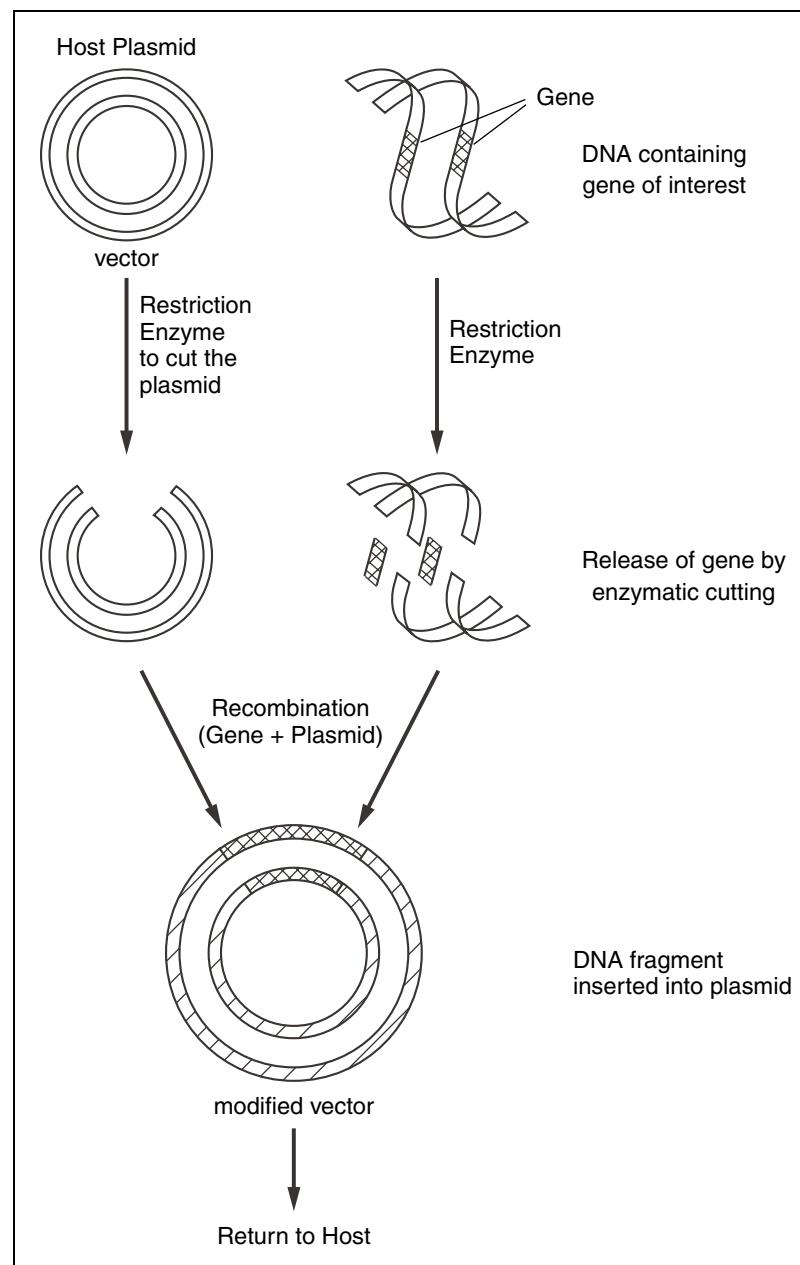


Figure 2 Gene cloning.

The basic steps of gene cloning first involve cutting a precise DNA segment (gene) from a donor source DNA by use of a restriction enzyme (Figure 2). At the same time, a small looped DNA (plasmid), a vector, from a host microorganism is snipped to open it up (Figure 2). The gene released from the donor is inserted (ligated) into the plasmid by a ligase, thereby closing the loop so that the plasmid is returned to its original state, except for the newly inserted gene (Figure 2). The modified vector is re-inserted into the host organism which replicates it (cloning). Large quantities of otherwise scarce proteins, corresponding to the newly inserted gene, can be synthesised by such cloning techniques, simply by growing and harvesting the modified microorganisms.

DNA Sequencing

DNA from a gene contains hundreds to thousands of nucleotide units for which the sequence is needed in order to interpret its code. Sequencing methods require only small amounts ($5\mu\text{g}$) of purified DNA, which can be produced by cloning. Automated sequenators are available which, daily, can sequence DNA containing hundreds of nucleotide units.

In the human cell there are 23 pairs of chromosomes, containing approximately 3000 million base pairs of DNA. Short sequences of DNA, perhaps with as few as 20 nucleotide units and sometimes radiolabelled, can be obtained either by chemical synthesis (gene machine) or from cloning. These short sequences can be used to 'probe' for a complementary sequence by looking for the position to which they bind to any DNA sample under investigation, from blood for example. Such probes can detect as little as 100fg of DNA, and are the basis of forensic genetic fingerprinting tests.

DNA sequence data have been used to investigate inherited diseases such as haemophilia and muscular dystrophy, and also in cancer research.

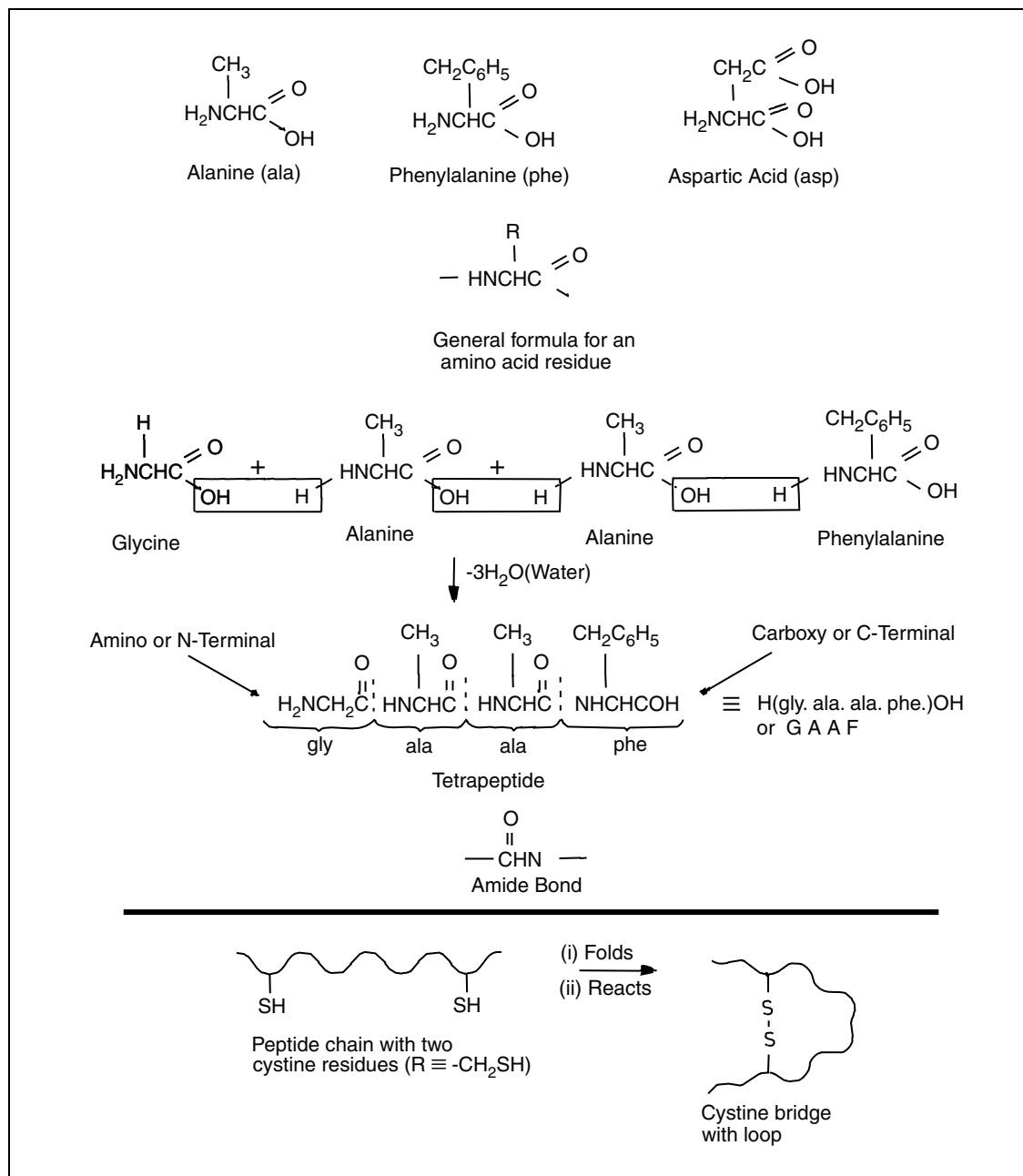


Figure 3 Formation of peptides and proteins

Proteins

Amino Acids, Peptides and Proteins

Amino acids (or strictly α -amino acids) are the building blocks of peptides, proteins and enzymes. Some examples (alanine, phenylalanine and aspartic acid) are shown in Figure 3. Peptides and proteins play key roles in most biological processes. Besides forming structural materials in animate species (hair, muscle, ligaments, etc.), other peptides and proteins and especially enzymes determine the pattern of chemical reactions in cells, and mediate many other functions such as transport and storage of nutrients, immune protection, and the control of growth. For example, haemoglobin transports oxygen in blood and the related myoglobin, transports oxygen in muscle; iron is carried in blood plasma by yet another protein, transferrin; ovulation is controlled by simple hormonal peptides; carbohydrates are broken down into sugars by enzymes.

Peptides and proteins are chain-like molecules made by the sequential linking of amino acids. Sometimes sugar molecules (glycosylates) or phosphate groups are attached at various points along the chain. For example, Figure 3 shows how a simple tetrapeptide can be constructed formally from four constituent amino acids, one each of glycine and phenylalanine and two of alanine, by elimination of water. The resulting tetrapeptide contains three amide linkages and can be named as H(gly.ala.ala.phe)OH, the three-letter abbreviations being used in place of the full name for each amino acid or GAAF if the one letter code is used. Note that the amino acid 'residues' in the chain are joined by amide bonds which give the peptide and protein chains a lot of physical and chemical strength (Figure 3).

Short chains of amino acid residues are known as di-, tri-, tetra-peptide and so on but, as the number of residues increases, the general names oligopeptide and polypeptide are used. When the chains grow to hundreds, the name protein is used. There is no definite point at which the name polypeptide is dropped for protein.

Twenty common amino acids appear regularly in peptides and proteins of all species. Each has a distinctive side chain (R in Figure 3) varying in size, charge and chemical reactivity.

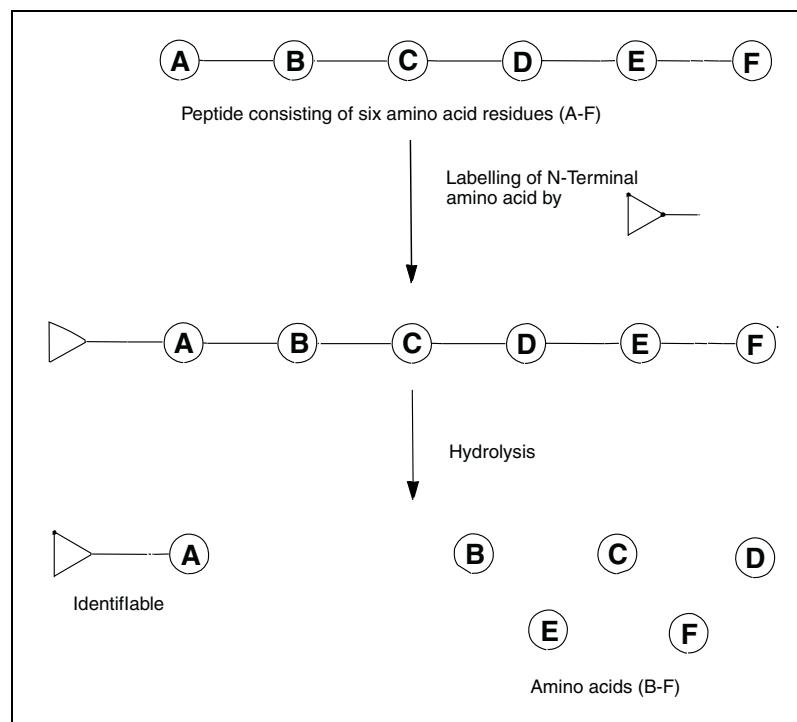


Figure 4 Identification of the amino-terminal residue of a peptide.

The sequence of amino acids in a peptide can be written using the three-letter code shown in Figure 3 or a one-letter code, both in common use. For example, the tripeptide, ala.ala.phe, could be abbreviated further to AAF. Although peptides and proteins have chain-like structures, these seldom produce a simple linear system but rather, the chains fold and wrap around each other to give complex shapes. The chemical nature of the various amino acid side groups dictates the way in which the chains fold to arrive at a thermodynamically most favoured state.

In enzymes, this folding process is crucial to their activity as catalysts, with part of the structure being the centre of reactivity. Heating enzymes (or other treatments) destroys their three dimensional structure and so stops further action. For example, in wine-making, the rising alcohol content eventually 'denatures' the enzymes responsible for turning sugar into alcohol and fermentation stops.

Peptides and proteins can be purified by a multiplicity of techniques. They can be separated from small molecules by dialysis through semi-permeable membranes, or by gel-filtration chromatography. They can be separated from each other by ion-exchange chromatography or by electrophoresis and its variants. The high affinity of many proteins for specific chemical groups is used to advantage for their separation and purification in affinity chromatography.

Sequencing Methods

The sequence of each different peptide or protein is important for understanding the activity of peptides and proteins and to enable their independent synthesis, since the natural ones may be difficult to obtain in small quantities. To obtain the sequence, the numbers of each type of amino acid are determined by breaking down the protein into its individual amino acids using concentrated acid (hydrolysis). For example, on hydrolysis the tetrapeptide shown in Figure 3 would give one unit of glycine, two units of alanine and one unit of phenylalanine. Of course, information as to which amino acid was linked to which others is lost.

The N-terminal residue of a peptide (gly in the tetrapeptide of Figure 3) can be identified by bonding another 'flag' molecule at this position, and then removing the modified residue from the peptide by hydrolysis (Figure 4). Unfortunately, this technique destroys the rest of the sequence and so the 'Edman' technique was developed to sequentially remove one amino acid at a time but, leaving the remainder of the chain intact.

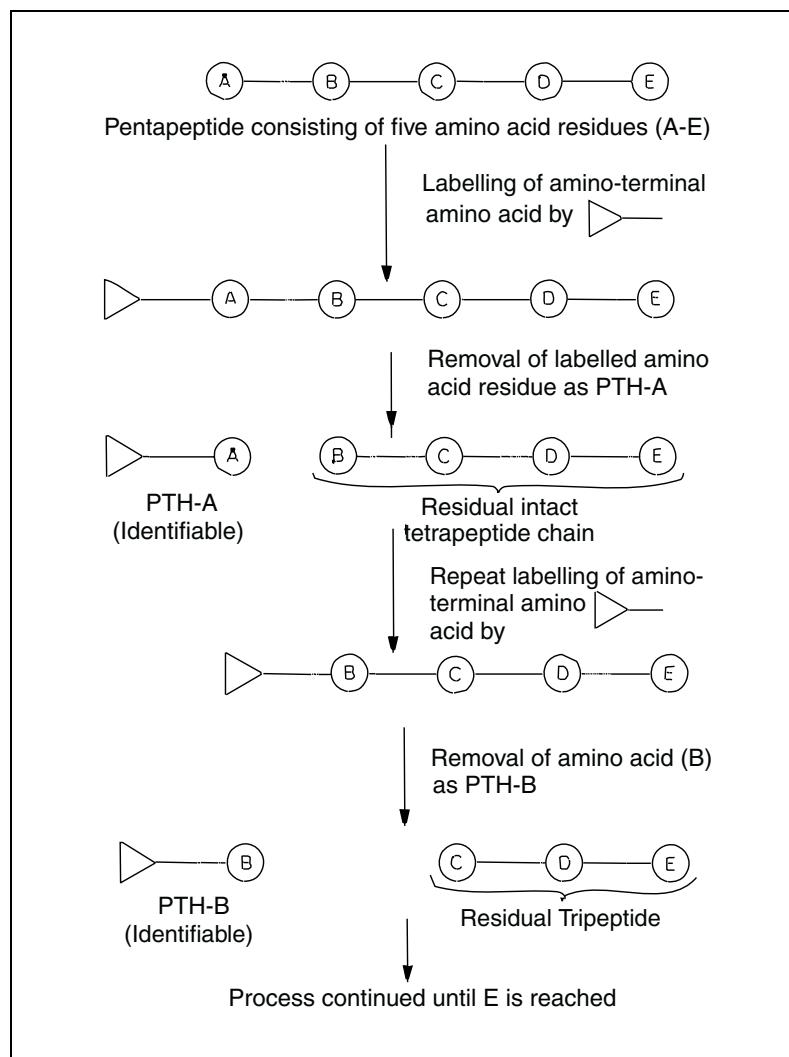


Figure 5 The 'Edman' sequential degradation of a peptide or protein.

Reaction with phenylisothiocyanate generates a phenylthiohydantoin (PTH) derived from the N-terminal amino acid (Figure 5). The terminal residue can be identified, leaving the remainder of the peptide intact so that the procedure can be iterated until the entire sequence has been determined.

Such chemical approaches to obtaining amino acid sequences have been fully automated and can be used with extremely small quantities of peptide or protein. They are frequently referred to as 'wet' methods because they are done in solution. Although a sensitive technique, the method begins to fail after about 20 to 40 residues have been determined and so it is necessary to break proteins into smaller peptide units. The Edman degradation is then used on the smaller fragments of the original chain. Although the amino acid sequences of these peptides are obtained, the order in which these smaller segments occur in the original protein is not defined. (Compare the result of cutting a ribbon without identifying which cut end goes with which other). The necessary additional information is obtained by creating 'overlap' peptides from treatment of the original protein with different chemicals or enzymes (cutting the ribbon at different points). This 'peptide mapping' can be used to determine the whole of a protein sequence containing hundreds of amino acids (Figure 6).

As mentioned above, automated sequencers are available and these can carry out complete Edman degradations on polypeptides containing tens of amino acids in less than one day.

An alternative approach to peptide sequencing uses a 'dry' method in which the whole sequence is obtained from a mass spectrum, thereby obviating the need for multiple reactions. Mass spectrometrically, a chain of amino acids breaks down predominantly through cleavage of the amide bonds, similar to the result of chemical hydrolysis.

From the mass spectrum, identification of the molecular ion, which gives the total molecular mass, followed by examination of the spectrum for characteristic fragment ions representing successive amino acid residues, allows the sequence to be read off in the most favourable cases.

However, interpretation of, or even obtaining, the mass spectrum of a peptide can be difficult and many techniques have been introduced to overcome such difficulties.

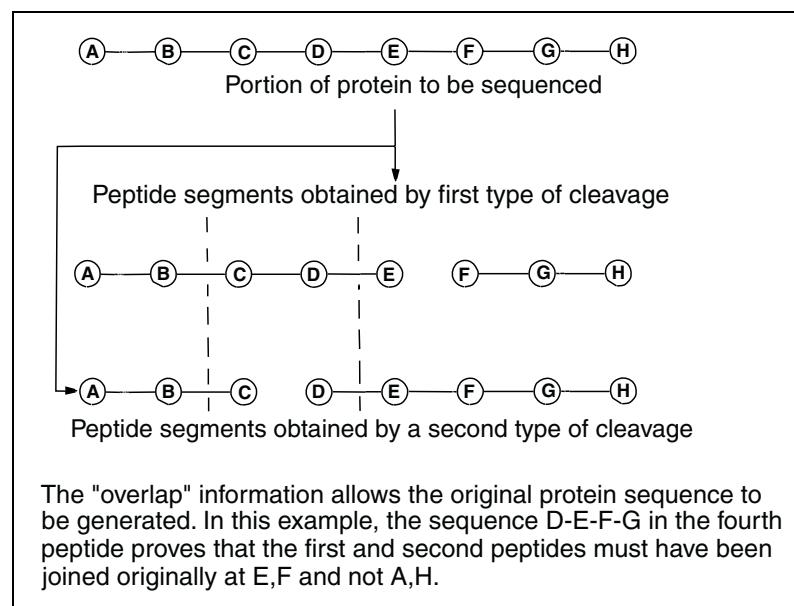


Figure 6 Protein sequencing by creation of 'overlap' peptides (mapping).

These techniques include modifying the side-chains in the peptide and protecting the N-and C-terminals by special groups. Despite many advances made by these approaches, it is not always easy to 'read off' the sequence from the mass spectrum because some amide bond cleavages are less easy than others and give little information.

To overcome this problem, *tandem mass spectrometry* has been applied to this 'dry' approach to peptide sequencing with considerable success. Further, *electrospray ionization* has been used to determine the molecular masses of proteins and peptides with unprecedented accuracy

Enzymes Enzymes have been touched upon already in various sections and here some of their uses outside of natural metabolism are touched upon.

Enzymes are proteins which catalyse specific chemical reactions in biological systems by binding to (enzyme) specific substrates. Chemical modifications of the substrate take place on the enzyme and the products are released. Because of their specificity in reaction, enzymes are being used increasingly in the chemical industry to catalyse chemical reactions, often to produce chiral compounds, which are difficult to isolate by conventional methods. Other uses of enzymes are developing and the technique of gene cloning allows large quantities of enzymes to be produced by utilising harmless bacteria as biochemical factories. As a final note, if the gene corresponding to a particular enzyme can be identified, it is no longer necessary to sequence the enzyme chain by identifying the amino acids directly. Instead, the sequence of nucleotides in the DNA is found and then, from the succession of codons, the complete sequence of the enzyme can be read off.

Conclusion Deoxyribonucleic acids are the chemical codes for genes, which are grouped together in chromosomes. The coding directs cellular synthesis in an organism, to give a wide variety of peptides, proteins and enzymes which, in turn, form structural materials (hair, tissue etc.), other chemicals for breaking down food (needed for growth and energy), and yet others for protecting the organism (immune response). All these processes can be interfered with naturally (mutation, disease) or artificially (genetic engineering).

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Back to Basics Section G: Applications

CHAPTER G4

CHOICE OF MASS SPECTROMETER

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Introduction

Early in the history of mass spectrometry, instruments were built by scientists on an individual basis and, often, could not be used or understood by anyone other than the people who had assembled them. Nowadays, very few scientists build their own mass spectrometers but many commercial types are available. Although commercial mass spectrometers can be adapted to specialised needs, in the large majority of cases, they are used by people who want fast, accurate answers to a wide range of questions such as, "Is this painting a genuine old one or is it a modern fake?" or, "Was this horse doped when it won the race?" or, "What is the structure of this new protein?". These last questions may be broadly classified under the heading of analysis, a topic which embraces a large proportion of use of commercial mass spectrometers. Because of this wide usage and the complex range of commercial mass spectrometers that are available, it is sometimes difficult to reach a decision when faced with buying such an instrument. This present Back-to-Basics guide is designed to present some of the factors that need to be considered if a reasoned decision is to be reached. However, any final decision may well be a compromise between what is desirable and what is available within any given price range. Items discussed in this present guide can be read in greater detail in the full Back-to-Basics set. For example, the theory and practical uses of electron ionisation are fully discussed in the guide *Electron Ionisation* and not here in the present guide. Some important factors to be considered in reaching a decision are discussed below.

**Objectives in buying
a mass
spectrometer**

The main objective of buying a mass spectrometer may seem to be a very obvious first step but it is an important one for achieving a satisfactory result. Before approaching suppliers of commercial mass spectrometers, it is wise to set out on paper exactly the analytical requirements for both the immediate and near future. The speed of advance in science and, especially in analysis and mass spectrometry, means that long range prognostications of future requirements are likely to be highly speculative and therefore of little relevance.

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Once basic requirements have been set out, together with other secondary objectives, the prospective purchaser will find it easier to discuss details with commercial sales representatives. From the latter's viewpoint, it is easier to talk to a potential customer who knows exactly or closely what he or she needs from a mass spectrometer system rather than to a customer who has only a vague idea of what is required. In fact, this last situation can lead to one of two extremes. Either a very expensive instrument may be purchased that is far too good for the analyses required or a very cheap instrument is acquired, which is inadequate for immediate needs let alone ones that might arise in the near future.

Future usage of a new instrument is worth considering, even if long range forecasting is unlikely to be useful. It is almost invariably the case that, once a new instrument has been installed and is operating routinely, people or groups within an organisation that has just acquired a new mass spectrometer begin to hear about what it can do. This leads to them thinking of new ways for carrying out analyses with the help of the new instrument. Such healthy developments often lead to pressure on available time on the new machine and an instrument that cannot accommodate some increase in output leads to disappointment and, often a need to make another purchase sooner than expected. Therefore, when contemplating the purchase of a new instrument, it is useful to explore the possibility that the machine may need to be adapted to increase throughput or to change the way it operates. For example, it may be desirable to fit an alternative type of ion source and an instrument that cannot be adapted easily, may be a drawback. These are relevant issues to raise with a sales representative, if only to set boundaries in the putative purchaser's own mind. Some instruments come as a complete system aimed at one primary objective and are almost impossible to alter, even modestly (unless expense is no object!). Other instruments may place an unacceptably low limit on the number of samples that can be examined per hour or per day.

Setting out the objectives in a detailed fashion and assigning an order of priority to those objectives is an absolute first essential step and is time well spent. Once carefully considered, it could become clear that the objectives fall into two different categories and that it might be cheaper to purchase two "dedicated" instruments rather than one large, all encompassing mass spectrometer.

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Types of sample

Samples may be single substances, complex mixtures of well-known, relatively simple substances or complex mixtures of substances of totally unknown structure or combinations of such analyses. It is impossible to generalise in such situations but it is possible to offer guide lines on some of the important issues.

For the simpler analyses, it may only be necessary to look for the presence of impurities in a “single” substance, which is volatile, thermally stable and easy to handle. Such analyses can be tackled with most kinds of mass spectrometer having simple inlet systems. At the other end of the scale of difficulty there are several major analytical pathways. The sample may be a “single” substance but be very intractable. For example, ceramics or bone are often thought of as single entities whereas, chemically, each is complex, involatile, difficult to break down into simpler identifiable species and presents severe analytical problems. The sample could be a mixture of polar, thermally unstable proteins or peptides from a biological sample, many of the constituents being of unknown structure. Then, the whole sample needs chromatography, a specialised inlet system and possible MS/MS facilities. As in the case of toxic substances, the important components of a sample may be present only as traces and high sensitivity of detection is needed.

Complexity of sample

“Complex” may refer to a sample in two ways. The sample may be a single substance but be very complex in chemical structure or the sample may be complex because it consists of a lot of different substances of varying polarity, volatility and thermal stability.

If samples are largely pure, single substances then the sample inlet may be quite simple, as with a direct insertion probe or a gas inlet. However, most analyses require assessment of the number of components, their relative proportions and their chemical structures. This level of complexity will normally need some degree of separation of the components before examination by mass spectrometry. Isolation and concentration of the components that need to be examined by mass spectrometry is usually a first step and, when properly designed, this sort of pre-analytical step can lead to big improvements in overall sensitivity and to less demand on the mass spectrometer. For example, volatile organic components from soil samples are usually removed by vaporization before analysis rather than trying to put the soil sample itself into the mass spectrometer.

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After this pre-separation stage (sometimes called sample “clean-up”), it may still be necessary to effect some separation of the required sample components. For example, with regard to the soil sample mentioned above, the isolated volatile organics may consist of literally hundreds of components present in widely varying amounts. In these circumstances, it is necessary to use a chromatographic procedure to separate the components before they are passed into the mass spectrometer. This can be done manually in that each component may be trapped as it emerges from a GC or LC column and then transferred to a simple inlet system on the mass spectrometer. It is much more likely that the GC or LC apparatus will be linked directly to the mass spectrometer so that, as each component emerges from the chromatographic column, it is transferred immediately into the mass spectrometer. Therefore, combined GC/MS or LC/MS or CE/MS may be needed, depending on the nature of the substances to be analysed. While there is some overlap, in that some substances could be examined by, say, GC/MS or LC/MS, it is usually the case that analyses are required using only one type of inlet. This is an important consideration because inlets for GC/MS, LC/MS and CE/MS are different and, if any two or all three of the inlets are necessary, the cost of the mass spectrometer system will be increased substantially. If changing from one inlet system to another is time-consuming, this will lead to “down-time” when the mass spectrometer cannot be used and this down-time can be costly for a busy analytical laboratory. This is another reason for considering carefully just what inlet systems are essential and which would be merely “useful”.

It might be noted at this stage that some mass spectrometer inlets are also ionisation sources. For example, with ES and AP_{Cl}, the inlet systems themselves also provide the ions needed for mass spectrometry. In these cases, the method of introducing the sample becomes the method of ionization and the two are not independent. This can be an important consideration. For example, with electrospray, abundant protonated molecular ions but no fragment ions are produced. While this is extremely important for accurate mass information and for dealing with mixtures by MS/MS, the lack of fragment ions gives almost no chemical structure information. If such information is needed then the mass spectrometer will have to be capable of fragmenting the protonated molecular ions through incorporation of a collision gas cell or other means, again adding to cost.

Table I. Complexity of substance to be examined and mass spectrometer type.¹

Complexity	Matrix assisted	GC/MS	LC/MS	CE/MS	MS/MS
Single substances	Any				✓
Mixture of volatile substances	Any	✓			✓
Mixture of solids in solution	Any		✓	✓	✓
Mixture of solids (involatile)	Any				✓

¹These generalisations indicate useful combinations of techniques for dealing with analyses of simple substances to complex mixtures. Observations on ionisation/inlet types appear in Tables 1-3. MS/MS can be used for examining mixtures with or without prior separation into individual components.

Prior separation of mixtures into individual components may not be needed. If the mass spectrometer is capable of MS/MS operation, one half of the mass spectrometer is used to isolate individual ions according to m/z value and the other half is used to examine their fragmentation products so as to obtain structural information.

For analytical laboratories having high throughputs of samples, it is usually necessary to have automatic samplers so that the mass spectrometer can work for 24 hours per day, even in the absence of an operator. Therefore, one consideration that may be important in deciding on an instrument could be as simple as asking how long it takes to set the operating characteristics of the mass spectrometer. How easy is it to calibrate? Most modern systems utilize computer programmes that automatically check and adjust voltages, peak shapes and so on at the touch of a button and calibration for accuracy of m/z value may be trivial but can also be complex, especially at high resolution.

Table I indicates which arrangement of mass spectrometer might be used for various sample types.

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Sample volatility, polarity and thermal stability

(i) Gases

The ease of vaporisation of a sample can be an overriding factor in choice of mass spectrometer. Broadly, the three phases gas, liquid and solid need to be considered. By definition, a gas is "volatile" and inserting a gas into a mass spectrometer is generally easy. A simple system of valves and filters is sufficient for transferring a gas into the vacuum of a mass spectrometer and, often, a straightforward EI source is all that is needed. Additionally, gases tend to be of low molecular mass and therefore ion analysers for gases do not need to be very sophisticated or to have more than a modest resolving power to cover the range needed (often less than an upper limit of m/z 100-150). Mass spectrometers for such purposes can be very small and light and for this reason were chosen to be carried on space probes to other planets. Similarly, such small mass spectrometers (usually quadrupole instruments) can be used to monitor atmospheres on earth in places where noxious substances may be present. These small mass spectrometers can be used in and transported by small vans or cars.

Apart from substances that are gaseous at normal ambient temperatures, other materials cross the divide between gas and liquid in the range when they have boiling points from -10 to +40 °C. Such substances are so volatile that their mass spectrometric analysis becomes just as easy as that for gases. For example, butane has a boiling point just below normal ambient temperatures and diethyl ether has a boiling point not much above most ambient temperatures. This intermediate volatility produces a grey area from the viewpoint of mass spectrometric analysis since the cut-off between a gas such as argon and a highly volatile liquid such as petrol is not sharp and is highly dependent on ambient or operating temperatures. Even some solids produce significant vapour pressures so that they could be analysed by simple "gas" mass spectrometers, if the mass range is sufficiently large. Camphor is a solid at normal ambient temperatures but it volatilises very easily (sublimes). It is worth recalling in this context that, usually, whatever the ambient atmospheric temperature, the mass spectrometer itself will not normally operate outside certain limits. Above about 35 °C, the electronic components in computer-controlled systems becomes increasingly unstable and frequently it is necessary to install suitable air-conditioning. Below about 10 °C, condensation of atmospheric moisture onto a mass spectrometer can lead to electrical problems through short circuits. Therefore, in considering whether or not a mass spectrometer would

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be useful for volatile liquids, it is worthwhile considering the temperature of the operating mass spectrometer and not simply outside ambient temperatures. A mass spectrometer operating in the Arctic or the Antarctic requires air conditioning just as much as one operating near the equator.

Of course some substances are sufficiently volatile that a heated inlet line can be used to get them into a mass spectrometer. Even here, there are practical problems. Suppose a liquid or solid is sufficiently volatile that heating it to, say, 50 °C is enough to get the vapour into the mass spectrometer through a heated inlet line. If the mass spectrometer analyser is at 30 °C, there is a significant possibility that some of the sample will condense onto the inner walls of the spectrometer and slowly vaporize from there. If the vacuum pumps cannot remove this vapour quickly then the mass spectrometer will produce a background spectrum of the substance added through the heated inlet line throughout successive analyses of other substances. This is the well-known "memory effect", which can be both tedious to remove and can slow down the throughput of analyses. For such reasons, it is probably best to consider that any substance that remains a liquid above about 50-70 °C should not be considered for analysis in a "gas" mass spectrometer. Similarly, it would probably not be wise for the most part to insert sublimable solids into such instruments.

(ii) Liquids

As with gases, there are no sharply defined limits for what should be considered a liquid and the best guide with regard to use of mass spectrometers probably comes from the operating temperature range (10—30 °C) of the instrument or any associated apparatus. A mass spectrometer inlet may be at atmospheric pressure or it may be under a high vacuum (10^{-5} — 10^{-6} mms of mercury). Clearly, introduction of a low boiling liquid into the inlet of a system under high vacuum will lead to its rapid volatilisation. It may even be that the pressure rise resulting from volatilisation of a liquid becomes so great that the instrument shuts itself down to safeguard its vacuum gauges and ion detectors. It is worth recalling that even substances with a boiling point of 100 °C or more will evaporate rapidly in a vacuum of 10^{-5} mms of mercury. The higher the boiling point, the less this is a problem. Liquids and even solids may well arrive at the mass spectrometer inlet in vapour form, as when they come from a GC chromatograph. Generally, the amounts of such emerging substances are very low so that, if the vacuum is high, transfer from the chromatographic column is easy through a heated line to the inlet or ion source of a mass spectrometer.

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Liquids that are sufficiently volatile to be treated as gases (as in GC) are usually not very polar and have little or no hydrogen bonding between molecules. As molecular mass increases and as polar and hydrogen bonding forces increase, it becomes more and more difficult to treat a sample as a liquid with inlet systems such as EI and CI, which require the sample to be in vapour form. Therefore, there is a transition from “volatile” to “non-volatile” liquids and different inlet systems may be needed. At this point, LC begins to become important for separation and connection to a mass spectrometer.

To achieve sufficient vapour pressure for EI and CI, a relatively involatile liquid will have to be heated strongly but this heating may lead to its thermal degradation. If thermal instability is a problem then inlet/ionisation systems need to be considered, which do not require pre-volatilisation of the sample before mass spectrometric analysis. This problem led to the development of inlet/ionisation systems that could operate at atmospheric pressure and ambient temperatures. Successive developments introduced the techniques of FAB, FIB, dynamic FAB, thermospray, plasmaspray, electrospray and APcl. Only the last two are now in common use. Further aspects of liquids in their role as solvents are considered below.

(iii) Solids

Substances that are solid at ambient temperatures are likely to have strong polar, electrostatic and sometimes hydrogen-bonding forces. In some solids such as sterols, these forces are sufficiently weak that they can be vaporised relatively easily and analysed by GC or GC/MS. They also are fairly stable to heat. However, many other solids such as proteins, carbohydrates or oligonucleotides have very strong electrostatic, polar and hydrogen-bonding forces and attempting to volatilise these substances using heating simply leads to their decomposition. Such materials need special methods for getting them into a mass spectrometer, especially if they are in solution in aqueous solvent, as is often the case. Therefore, while some solids can be examined by GC/MS methods or even by simple introduction probes by EI or CI (perhaps after suitable derivatisation), many other solids need to be inserted into a mass spectrometer in a different manner, such as by EI or APcl.

Solutions of solids may need to be examined by converting them into aerosols by pneumatic or sonic spraying techniques. After solvent has evaporated from the aerosol droplets, the residual particulate solid matter can be ionised by a plasma torch.

Table 2. Molecular type and mode of ionisation¹

Molecular type²	EI	CI	ES	APCI	MALDI	PT	TI
Gas	✓	✓				✓	
Liquid (volatile)	✓	✓					
Liquid (involatile)			✓	✓		✓	✓
Solid (volatile)	✓	✓			✓	✓	
Solid (involatile)			✓ ³	✓ ³	✓	✓	✓
Solution (direct insertion)			✓	✓		✓	✓

¹ EI = electron ionisation; CI = chemical ionisation; ES = electrospray; APCI = atmospheric pressure chemical ionisation; MALDI = matrix assisted laser desorption ionisation; PT = plasma torch (isotope ratios); TI = thermal (surface) ionisation (isotope ratios).

² These are only approximate guides.

³ Solids must be in solution.

Some solid materials are very intractable to analysis by standard methods and cannot be easily vaporised nor dissolved in common solvents. Glass, bone, dried paint and archaeological samples are common examples. These materials would now be examined by laser ablation, a technique that produces an aerosol of particulate matter. The laser may be used in its defocused mode for surface-profiling or in its focused mode for depth-profiling. Interestingly, lasers can be used to vaporise even thermally-labile materials through use of the MALDI variant.

For solids, there is now a very wide range of inlet and ionisation opportunities so that most types of solids can be examined, either neat or in solution. However, the inlet/ionisation methods are often not simply interchangeable, even if they utilise the same mass analyser. Thus, a direct insertion probe will normally be used with EI or CI (and DCI) methods for ionisation. An LC will be used with ES or AP_CI for solutions and nebulisers may be used with plasma torches for other solutions. MALDI or laser ablation are used for direct analysis of solids.

Table 2 indicates which ionization mode is likely to be most suitable for different physical properties of sample substances.

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Mass analysers

Commercial mass analysers are almost entirely based on quadrupoles, magnetic sectors (with or without an added electric sector for high resolution work) and time-of-flight configurations or a combination of these. There are also ion traps and ion cyclotron resonance instruments. These are discussed as single use and combined (hybrid) use.

(i) Single analysers

The advantages and disadvantages of these analysers need to be examined within the context for which they will be used, rather than for their overall attributes. For example, a simple quadrupole, which is robust and can examine substances at unit mass resolution easily up to m/z 200 would be ample for most gas analysers but would be totally unsuitable for accurate mass measurement in most applications. Conversely, a magnetic sector instrument of 50,000 resolution and capable of measurement to m/z 100,000 would be totally wasted for the previously mentioned gas analysis. Since the difference in cost would be very large, there would be no difficulty in making a choice. However, just as defining boundaries between gases, liquids and solids is a tenuous exercise in the face of considerations of vacuum, volatility and thermal stability so the choice of mass analyser can become a difficult one.

(ii) Resolving power and mass range

The ability of an analyser to effect unit resolution of m/z values is important. It is useful to differentiate a "working" resolution as against a "best" resolution. For obvious reasons, a mass spectrometer manufacturer will want to quote the "best" measured resolution attainable on any one instrument but the purchaser needs to remember that this measurement will have been obtained when everything is working perfectly. Under everyday conditions, the mass spectrometer is unlikely to be operating even close to this best limit and therefore effective resolving power is likely to be much lower than the best. As a rough guide, it is probably not unreasonable to subtract say 10-15% from "best" resolution figures to give some idea of the "effective" resolution obtainable under normal working conditions. Even then, choice of analyser is not necessarily easy. As a general guide, up to m/z 600-1000 with unit mass resolution, all types of analyser will be sufficient but the quadrupole or ion trap is likely to be cheapest. As the working range increases, the quadrupoles and ion traps begin to drop out of consideration and time-of-flight or sector instruments come to the fore. Of these, the time-of-flight is good up to m/z 3000-5000, although the higher mass ranges would probably need to include the use of a reflectron. As with the quadrupoles and ion traps, the time-of-flight instruments are robust and easy to

Table 3. Typical mass ranges achievable with various matrix assisted.

M/z range¹	Quadrupole	TOF	Sector	Ion trap	ICR
0-1500	✓	✓	✓	✓	✓
1500-3000		✓	✓		✓
3000-30,000			✓		✓
30,000-100,000			✓		✓
> 100,000			3		✓

¹ Most ion sources produce singly-charged ions, i.e., $z = 1$ and the ranges shown here apply to such ions. Matrix assisted measure m/z values, not mass directly. When $z = 1$, $m/z = m$, viz., mass can be measured directly. An ES ion source produces ions with $z > 1$ and this effectively extends the mass ranges that can be examined. For example, with $z = 1$ and $m = 10,000$, the m/z value is 10,000 and this would be beyond the capabilities of a quadrupole instrument. However, with $z = 10$ and $m = 10,000$, $m/z = 1000$ and this value is well within the range of a quadrupole analyser.

operate. An added consideration is that the time-of-flight instruments are particularly easy and quick to calibrate. As the mass range increases again (m/z 5000-50,000), magnetic sector instruments (with added electric sector) and ion cyclotron resonance instruments are very effective but their prices tend to match the increases in resolving powers. At the top end of these ranges, masses of several million have been analysed by using Fourier Transform ion cyclotron resonance instruments but such measurements tend to be isolated rather than everyday achievable targets.

Simple considerations of achievable mass resolution and measurement become unrealistic when using electrospray methods of ionisation because m/z values change markedly. A substance of mass 10,000 with a single positive charge has an m/z value of 10,000 and this is measurable easily with a magnetic/electric sector combination but not with a quadrupole or a time-of-flight instrument. However, a substance of mass 10,000 but with 10 positive charges has an m/z ratio of only 1000 and this can be measured with a quadrupole, ion trap or time-of-flight instrument. Simply by changing from one ionisation method to another can reduce or increase the demands on mass resolving power from "relatively cheap" to "expensive" or vice versa.

For accurate mass measurement, high resolving power is necessary. The difference in degrees of difficulty between measuring an m/z of 28 and one of 28,000 is likely to be very large. Broad mass ranges achievable with various analysers are shown in Table 3.

(iii) Combined analysers (hybrid instruments)

For some kinds of analyses, it is convenient to have two combined mass spectrometers. This naturally increases costs and therefore purchase of such hybrid instruments tends to require much more careful consideration of needs. Hybrid instrumentation is necessary for MS/MS purposes, for which the two mass spectrometers may be quadrupoles, magnetic sectors or a combination of these with each other or with time-of-flight analysers. Often the combination is linked via a hexapole or quadrupole "bridge", which serves to channel all ions from one MS to the other or the quadrupole may serve as a gas collision cell. For MS/MS purposes, ions selected by m/z value in one mass spectrometer are passed through a collision cell to induce fragmentation and the fragment ions are examined in the second mass spectrometer. Thus, in the "triple quad" or QQQ analyser, the central quadrupole is operated in such a way that no mass separation occurs but collisions of ions with gas can take place (this quadrupole mode is sometimes represented as q rather than Q, as in QqQ). Analysis by MS/MS is particularly easy in this combination, as is

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selected reaction monitoring, a technique for seeking out small traces of substances from other (mass spectrometrically) overlying (masking) substances. Two sector instruments linked together give a wide range of MS/MS techniques but at greater cost. The time-of-flight instrument (TOF) is frequently linked to a quadrupole (Q-TOF) for MS/MS purposes.

A major divergence appears for ion traps and ion cyclotron resonance (ICR) mass spectrometers. In both of these, MS/MS can be carried out without the need for a second analyser. The differentiation is made possible by the length of time ion traps or ICR instruments can hold selected ions in their mass analysers. For quadrupoles, magnetic sectors and time-of-flight analysers, the ions generated in an ion source pass once through the analyser usually in a time measured in a few microseconds. For ion traps and ICR instruments, ions may be retained in the trap or resonance cell for periods of milliseconds. In the latter case, it becomes possible to select ions at low background gas pressure, to collisionally activate these ions by increasing their velocity relative to background ("bath gas") gas for a short time and then to examine the resulting fragment ions. It is even possible to carry out MS^n analyses, where n may be from 2 to about 5. Ion traps, like quadrupoles, are limited in ultimate mass that can be measured but they provide a relatively cheap introduction to MS/MS. The ICR instruments tend to be as expensive as bigger hybrid instruments but can also be used in MS^n measurements.

Ionisation methods

At the beginnings of mass spectrometry as a routine analytical tool, the only commercial ion sources were EI. As needs have increased, not only have the types of matrix assisted increased and improved but more ionisation methods have appeared. Many different types of ionisation source have been described and several of these have been produced commercially. The present situation is such that there is now effectively only a limited range of ion sources. For vacuum ion sources, EI is still widely used but frequently in conjunction with CI. For atmospheric pressure ion sources, the most frequently used are ES, APCl, MALDI (lasers), plasma torches and TI (thermal ionisation).

(i) Electron ionization (EI) and chemical ionization (CI)

For gases and volatile liquids or solids, these methods are generally useful and give both molecular and fragment ions with relatively high efficiency. EI often results in relatively low yields of molecular ions compared with fragment ions. In contrast, CI gives high proportions of quasi-molecular ions to fragment ions, so that it is convenient to have a combined EI/CI source to allow easy, rapid switching between the two ionisation modes. Then, the molecular mass of a sample

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substance becomes clearly defined (CI) and its chemical structure may be inferred from the fragment ions (EI). Mass spectrometer manufacturers will normally offer such a combined EI/CE source. Switching between the two modes occupies only a second or two and the alternate switching from EI to CI and back can be accomplished within the time taken for a substance to emerge from a GC column.

The poor yield of molecular ions during EI is partly due to the inherent instability of some cation-radicals formed on ionisation. Since CI gives quasi-molecular ions that have been largely thermally equilibrated there is little fragmentation. However, both CI and EI require the sample to be in the gas phase and this means that thermal instability of the sample can be a serious problem. Thus, a substance such as toluene may be vaporised extremely easily under the vacuum conditions existing inside a mass spectrometer but sugars, proteins or oligonucleotides are involatile and degrade extensively on being heated to quite modest temperatures. Obtaining an EI or CI spectrum of toluene is very easy but polar, high molecular mass compounds will give only spectra characteristic of breakdown products (there are some instruments that make use of this thermal effect to investigate complex mixtures, as in Pyrolysis/MS or even Pyrolysis/GC/MS).

There are ill-defined limits on EI/CI usage based mostly on these issues of volatility and thermal stability. Sometimes these limits can be extended by preparation of a suitable chemical derivative. For example, polar carboxylic acids generally give either no or only poor yields of molecular ions but their conversion into methyl esters affords materials, which are much less polar and more volatile and can be examined easily by EI. In the absence of an alternative method of ionisation, EI/CI may still be used after clever manipulation of chemical derivatisation techniques.

There are methods for vaporising solids of low volatility by placing them on a thin wire, which is then raised to a high temperature within a fraction of a second (DCI). This rapid heating allows some vaporisation without decomposition but, with development of later ionisation methods, it is now rarely used.

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(ii) Electrospray ionization (ES) and atmospheric pressure chemical ionization (APCI)

These ionisation methods were initially developed to enable the effluent from an LC column to be passed directly to a mass spectrometer. It is necessary to remove the solvent from the effluent without removing too much of the solute itself and without application of too much heat. For ES, the effluent is sprayed at atmospheric pressure and temperature into a desolvation region, in which most of the solvent evaporates and the residual sample (solute) molecules pass into a matrix assisted, usually as protonated molecules. The sample does not need to be heated and the protonated (quasi-) molecular ions are thermally equilibrated by collisional processes during the evaporation of solvent. No fragment ions are formed. APCI is similar except that the method of spraying is different. Additionally, APCI uses another ionization section (corona discharge) to enhance the yield of protonated molecular ions.

Both ES and APCI afford good yields of stable protonated molecular ions. Because there is no fragmentation and therefore little structural information, it becomes necessary to add some internal energy to the molecular ions to cause them to decompose (this is collisionally induced decomposition or activation). This extra internal energy may be added simply by making the ion beam pass through a region having a modest density of collision gas present (helium or argon for example). Alternatively, the molecular ions may be speeded up in the desolvation region so that they collide with evaporating neutral solvent molecules. It may be noted that each of ES and APCI provides both an ion source and an inlet system for whatever matrix assisted is used (quadrupole, ion trap, magnetic sector, time-of-flight, ion cyclotron resonance).

Apart from ES and APCI being excellent ion sources/inlet systems for polar, thermally unstable, high molecular mass substances eluting from an LC or a CE column, they can also be used for stand-alone solutions of substances of high to low molecular mass. In these cases, a solution of the sample substance is placed in a short length of capillary tubing and is then sprayed from there into the mass spectrometer.

There is an added attribute of ES. Because the spraying process utilises application of a high positive or negative electric charge, the resulting protonated or other ions have more than one electric charge and this makes high molecular mass measurement much easier. For example, if a substance of mass 10,000 has 10 negative charges in its “molecular ion” then $m = 10,000$ and $z = 10$ so that m/z is equal to 1000. It is relatively easy to measure small m/z values, using mass spectrometers of modest resolving powers. Accurate

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measurement of large m/z values is much more demanding. Thus, ES provides a means for measuring large molecular masses with high accuracy.

(iii) Matrix assisted
laser desorption
ionization (MALDI)

A laser pulse lasting a few pico- or nano-seconds and focused into a small area can deposit a large amount of energy into a sample so that it is rapidly vaporised to form an aerosol. Unfortunately, most of the ablated material has no excess of electric charge and, unless a secondary ionisation source is used (see later), there would be very low sensitivity because of the low yields of ions. If the sample substance is intimately mixed with a suitable matrix material, the ion yield can be greatly enhanced. The matrix is usually a relatively volatile acidic substance that can absorb laser light efficiently. During vaporization following absorption of a laser pulse, the entrained sample substance is also vaporized. Since the sample is usually less acidic than the matrix, proton transfer occurs between them to give protonated molecular ions of the sample. As these ions are produced by a low energy proton transfer step, there is little fragmentation. This method is very good for producing molecular mass information from polar, thermally unstable high molecular mass biochemicals such as oligonucleotides, carbohydrates and proteins. These sorts of samples often need to be examined in large numbers and special auto-inlets (auto-samplers) are available. Once inserted into the instrument, the autosampler allows large numbers of samples to be examined routinely. Various matrix assisted may be used with the MALDI ion source. If structural information is needed, there must be some induced fragmentation of the protonated molecular ions and this is mostly provided by the use of gas collision cells, as with ES and APCL or by ion/molecule collision in an ion trap or ICR instrument.

(iv) Plasma torch (PT)
and thermal (surface)
ionisation (TI)

The above discussion has centred on how to obtain as much molecular mass and chemical structure information as possible from a given sample. However, there are many uses of mass spectrometry, in which precise isotope ratios are needed and total molecular mass information is unimportant. For accurate isotope ratio measurement, the sample must be vaporized and then directed into a plasma torch. The sample may be a gas or a solution that is vaporised to form an aerosol or it may be a solid that is vaporised to an aerosol by laser ablation. Whatever method is used to vaporise the sample, this is then swept into the "flame" of a plasma torch. Operating at temperatures of about 5000 K and containing large numbers of gas ions and electrons, the plasma causes any substance caught up in it to be totally fragmented into ionised atoms within a few milliseconds. The ionised

Table 4. Types of ions formed by various ion sources¹

Ionisation method	Type of molecular ion formed	Good molecular mass information	Abundant fragment ions	MS/MS needed for structural information	Accurate values for isotope ratios
EI	$M^{+\bullet}$, $M^{-\bullet}$	2	✓		
CI	$[M+H]^+$, $[M+X]^+$, $[M-H]^-$	✓		✓	
ES	$[M+H]^{n+}$, $[M-H]^{n-}$	✓		✓	
MALDI	$[M+H]^+$, $[M+X]^+$	✓			✓
APCI	$[M+H]^+$, $[M+X]^+$, $[M-H]^-$		✓ ³	✓ ³	✓
PT	$A^{+\bullet}$, $A^{-\bullet}$	✓	3	3	✓
TI	$A^{+\bullet}$, $(A^{-\bullet})$	✓ ³	3	3	✓

¹ The indications in this table are generalisations.

² Electron ionisation usually gives some molecular ions and sometimes these are abundant. Often it is difficult to be certain which ion, if any, is the molecular ion. For this reason, it is advantageous to obtain both EI and CI spectra, the first giving good structural information and the second good molecular mass information.

³ Plasma torches and thermal ionisation sources break down substances into atoms and ionised atoms. The latter are used for measurement of accurate isotope ratios. In the breakdown process, all structural information is lost, other than an identification of elements present (as in ICP/MS for example).

atoms are passed into a matrix assisted for measurement of their atomic mass and abundance of isotope. Even intractable substances such as glass, ceramics, rock and bone can be examined directly by this technique.

Since detailed chemical structure information is not usually required from isotope ratio measurements, it is possible to vaporise samples by simply pyrolysing them. For this purpose, the sample can be placed on a tungsten, rhenium or platinum wire and heated strongly in vacuum by passing an electric current through the wire. This is thermal or surface ionisation (TI). Alternatively, a small electric furnace can be used when removal of solvent from a dilute solution is desirable before vaporisation of residual solute. Again, a variety of matrix assisted can be used for the actual measurement of m/z values of atomic ions and their relative abundances.

Table 4 indicates suitable commonly available ionization sources for various classes of substance.

Overall view of choices

The above discussion has concentrated on major factors likely to be important in making a choice of which mass spectrometer is best for any defined purpose. Clearly, there are likely to be other issues that need to be taken into account and instrument price will be one of these. Some of the major considerations have been included here (Tables 1-4).

Conclusion

Choice of mass spectrometer to fulfil any particular task must take into account the nature of the substances to be examined, the degree of separation required for mixtures, the types of ion source and inlet systems and the types of matrix assisted. If these individual requirements have been defined, it is much easier to discriminate amongst the large number of commercial instruments that are available. Once suitable mass spectrometers have been identified, it is then often a case of balancing capital and running costs, reliability, ease of routine use, after sales service and reputation of the manufacturer.

Back to Basics Section G: Applications

CHAPTER G5

PEPTIDES AND PROTEINS ANALYSIS BY MS

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Quick Guide

- Amino acids are the molecular building blocks of peptides and proteins. About 20 common amino acids are known.
- Peptides and proteins are formed by linking successive amino acids into chains or rings. The order (sequence) and types of amino acids determine the chemical and physical properties of peptides and proteins.
- An enzyme is a special protein which acts as a catalyst for biochemical reactions.
- Fast Atom Bombardment (FAB) is an ionization technique which produces a protonated or deprotonated molecular ion and hence a molecular mass for the sample.
- Liquid Secondary Ion Mass Spectrometry (LSIMS) is a similar but more recent technique than FAB, and produces indistinguishable data.
- Dynamic/Continuous Flow FAB allows a continuous stream of liquid into the FAB source, and hence constitutes an LC-MS interface.
- An enzyme digest is the term applied to a process whereby a peptide or protein is mixed with a selected enzyme under favourable conditions to allow reaction to occur. The enzyme splits the peptide or protein into smaller units which are easier to identify.
- Post-translational modifications to proteins are biochemical in origin and alter the measured molecular mass relative to that calculated for an untranslated sequence.
- Laser Desorption Mass Spectrometry (LDMS) coupled to a Time-of-Flight analyser produces an unresolved protonated or deprotonated molecular ion cluster with virtually no upper mass limit.
- Electrospray (ES) produces a series of multiply charged ions which is transformed into an accurate molecular mass profile with virtually no upper mass range limit.
- Peptides and proteins can be analysed by mass spectrometry. Molecular mass information can be found by FAB or LSIMS for samples up to 10 kDa in mass. Laser Desorption and electrospray can analyse much higher molecular mass samples.

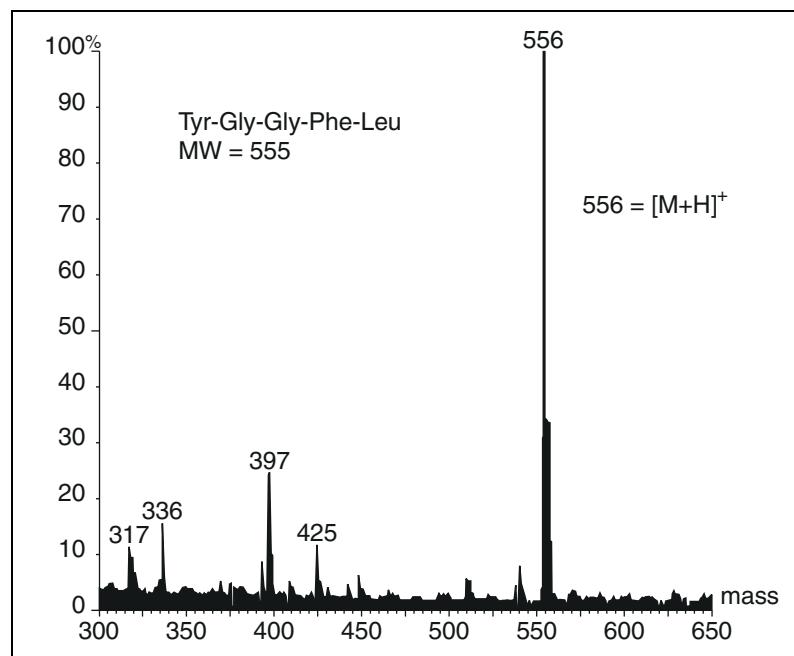


Figure I For this FAB experiment, a sample of the pentapeptidic enkephalin, Tyr.Gly.Gly.Phe.Leu., dissolved in glycerol was bombarded by xenon atoms. The resulting mass spectrum shows abundant protonated molecular ions at m/z 556.

- Tandem Mass Spectrometry (MS-MS) produces precise structural or sequence information by selective and specific induced fragmentation, routinely on samples up to 2500 Da. For samples of greater molecular mass than this, an enzyme digest will usually produce several peptides of molecular mass suitable for mass spectrometry.
- Samples containing mixtures of peptides can be analysed directly by electrospray. Alternatively they can be separated and analysed by LC-MS coupling techniques such as Dynamic/Continuous Flow FAB, or Electrospray.

Summary

The use of mass spectrometry for the analysis of peptides, proteins and enzymes has been summarized. This guide should be read in conjunction with others in the 'Back to Basics' series including 'Biotechnology' and those describing specific ionization techniques in detail.

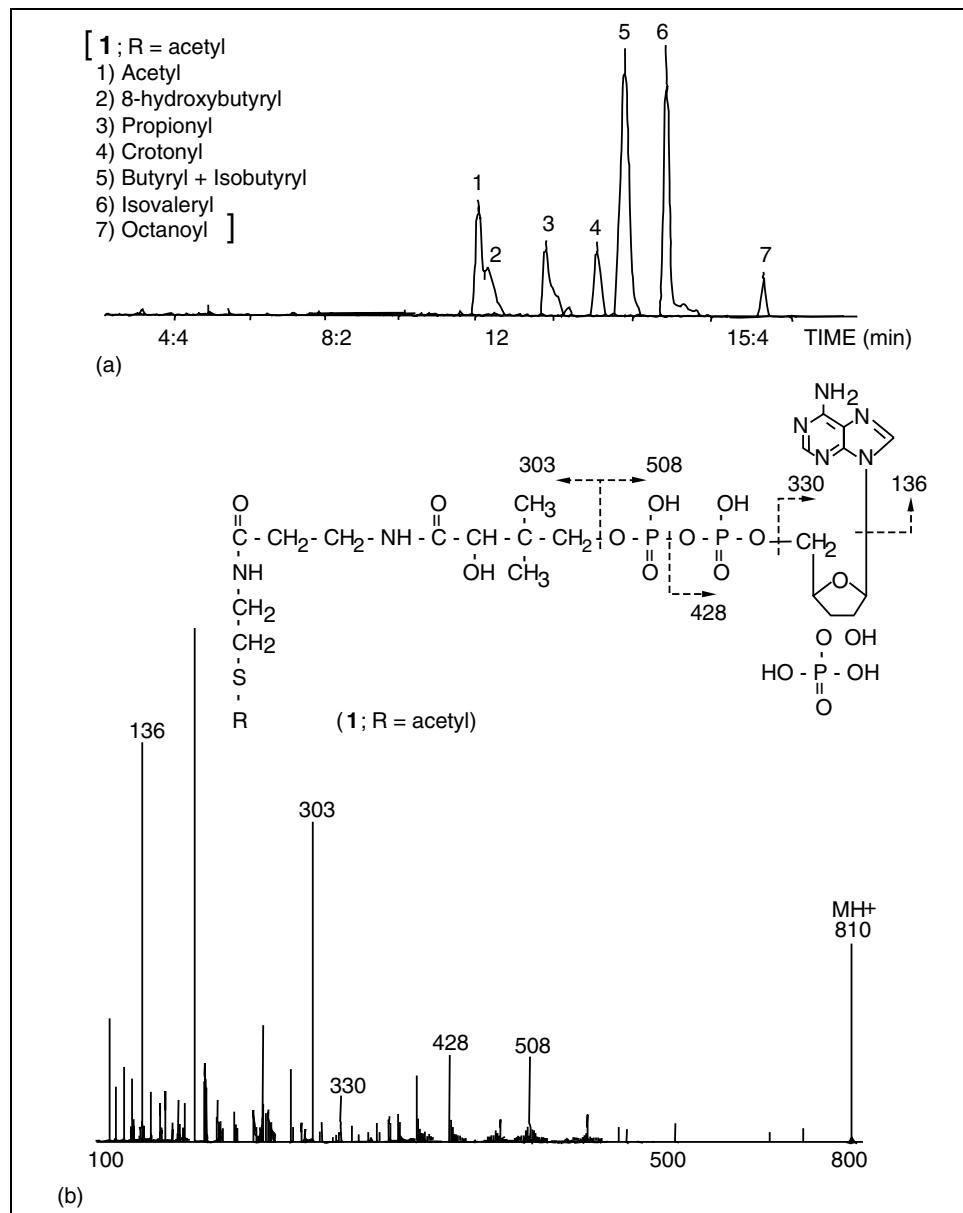


Figure 2 (a) LC-FAB-MS analysis of short and medium chain acyl coenzyme A compounds (**1**, = 1-7; 0.5 nmol of each). These compounds carry acyl groups for enzyme reactions and a number of metabolic diseases can be traced to enzyme deficiencies which result in the accumulation of toxic coenzyme A thioesters. (b) The FAB mass spectrum of acetyl coenzyme A (component **1.1** from the trace shown in (a)). The likely origin of major fragment ions is indicated.

ANALYSIS OF PEPTIDES AND PROTEINS BY MASS SPECTROMETRY

Introduction

Until 1981, mass spectrometry was limited, generally, to the analysis of volatile, relatively low molecular mass samples and was difficult to apply to involatile peptides and proteins without first cutting them chemically into smaller volatile segments. During the past decade the situation has changed radically with the advent of new ionization techniques and the development of tandem mass spectrometry. Now, the mass spectrometer has a well-deserved place in any laboratory interested in the analysis of peptides and proteins.

Fast Atom Bombardment (FAB)

The first breakthrough came with the development of FAB which enabled polar compounds of large molecular mass to be ionized without application of heat for volatilization of the sample.

In FAB (see 'Back to Basics' guide), the sample is dissolved in a suitable solvent (also called a matrix) of low volatility (e.g. glycerol, thioglycerol, m-nitrobenzyl alcohol) and is bombarded by a beam of fast xenon or argon atoms. Ionization produces protonated $[M+H^+]$ or deprotonated ($[M-H]^-$) molecular ions, sometimes accompanied by a little fragmentation. The matrix reproducibly gives background ion peaks but these can interfere with sample ion peaks and usually dominate the low mass end of the spectrum (Figure 1). Different samples exhibit different levels of response to FAB and, with a mixture of components, it is feasible that not all will be detected; in some cases, the minor components of a sample appear more prominently in the mass spectrum than the major ones.

Despite these limitations, FAB is in widespread use and is an excellent technique for determining the molecular masses of peptides up to 10,000 Daltons, with an accuracy of 0.5 Da.

FAB has evolved and fast atoms are being replaced by fast ions, such as Caesium (Cs^+). This variation is called Liquid Secondary Ion Mass Spectrometry (LSIMS) because the sample solution affords the secondary ion beam whilst the bombarding ions constitute the primary beam. Spectra produced by FAB and LSIMS are virtually identical, although higher sensitivity at high mass (10 kDa) is claimed for the latter.

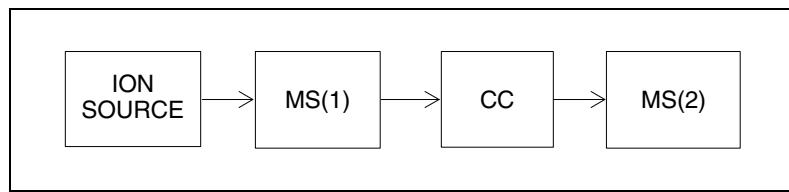


Figure 3 A typical MS-MS configuration. Ions produced from a source (e.g. dynamic-FAB) are analysed by MS(1). Molecular ions (M^+ or $[M+H]^+$ or $[M-H]^-$ etc.) are selected in MS(1) and passed through a collision cell (CC) where they are activated by collision with a neutral gas. The activation causes some of the molecular ions to break up and the resulting fragment ions provide evidence of the original molecular structure. The spectrum of fragment ions is mass analysed in the second mass spectrometer, MS(2).

Dynamic FAB

Another development arising from FAB has been its transformation from a static to a dynamic technique, by allowing a continuous flow of a solution to travel from a reservoir through a capillary to the probe tip. Samples are injected either directly, or through a liquid chromatography (LC) column. The technique is known as Dynamic or Continuous Flow FAB/LSIMS and provides a convenient direct LC-MS coupling for the on-line analysis of mixtures, (Figure 2). Mixtures, as with the acyl coenzyme A factors shown in Figure 2, can be separated and analysed on-line. In peptide and protein work, the peptidic substance is often reacted (digested) with enzymes which cleave the peptide or protein at places along its backbone to give smaller peptides. This digest (mixture of peptides) must be separated into its components and the newly-formed peptides identified so that the original structure can be deduced (this is called 'mapping' and is something like assembling a linear jigsaw). LC-FAB-MS is well-suited to the separation of such mixtures and the identification of components through their molecular masses. However, not only the molecular mass of a peptide is important. The actual sequence (order) of amino acid residues making up the peptide chain is also important and FAB, which gives predominantly molecular mass information and few structural pointers, must be supplemented by another technique, MS-MS or tandem MS.

Mass Spectrometry-Mass Spectrometry (MS-MS)

Typically, a sample is analysed by FAB-MS to obtain a relative molecular mass and then by FAB-MS-MS to achieve structural information by fragmenting the molecular ion and examining the fragment ions. This is achieved by passing the molecular ion from the first mass analyser into a collision cell (Figure 3). Here collision gas (e.g. argon) is used to fragment this ion. The fragment ions produced are analysed by a second mass spectrometer.

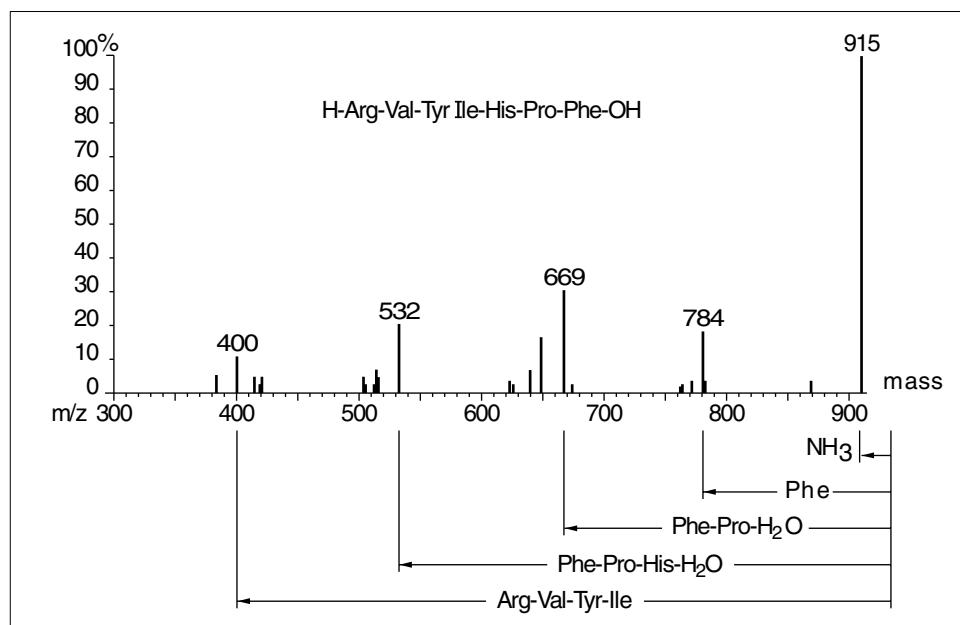


Figure 4 A typical FAB-MS-MS experiment on a peptide. The FAB-MS spectrum of angiotensin III is dominated by the protonated molecular ion $[M+H]^+$ at m/z 932, confirming the molecular mass of 931. In an MS-MS experiment only the ion at m/z 932 was allowed to pass through the first mass analyser into the collision cell (Figure 3). On passing through the collision cell several fragment ions were produced and were all analysed by the second mass analyser to produce the spectrum shown here. The ion at m/z 915 arises by loss of NH_3 from 932. The ions at m/z 784, 669, 532 and 400 arise respectively by loss of a phenylalanine residue; phenylalanine, proline and a water molecule; phenylalanine, proline, histidine and a water molecule; and arginine, valine, tyrosine and isoleucine, from 932. These fragments verify the expected sequence of amino acid residues in Angiotensin III.

The fragments can only arise directly from the molecular ion and so provide useful sequence information for peptides. Peptides have been found to fragment by predictable pathways along their backbone from the C-terminus and/or the N-terminus, as shown by the example of angiotensin III (Figure 4).

Tandem mass spectrometers most commonly used for MS-MS studies include the following analyser combinations, although many others are possible:

1. quadrupole - collision cell - quadrupole
2. magnetic/electrostatic analyser - collision cell - quadrupole
3. magnetic/electrostatic analyser - collision cell
- magnetic/electrostatic analyser

The collision cell used with the first two types (1, 2) is an RF-only quadrupole or a hexapole. This type of cell adds only a relatively small amount of energy to an ion during its collision with the cell gas. The third type uses a high energy collision cell which has the advantage of producing fragmentation of amino acid side chains as well as the 'backbone' fragmentation shown in Figure 4. This extra fragmentation gives information which permits differentiation of the two isomeric amino acid residues, leucine and isoleucine. Sequence information has been obtained by MS-MS on samples up to 2500 Da, which covers most enzyme digest generated peptides, often at low pmole levels, and usually in just a few minutes.

Other Ion Sources

The techniques described thus far cope well with samples up to 10 kDa. Molecular mass determinations on peptides can be used to identify modifications occurring after the protein has been assembled according to its DNA code (post-translation), to map a protein structure, or simply to confirm the composition of a peptide. For samples with molecular masses in excess of this, the sensitivity of FAB is quite low and such analyses are far from routine. Two new developments have extended the scope of mass spectrometry even further to the analysis of peptides and proteins of high mass.

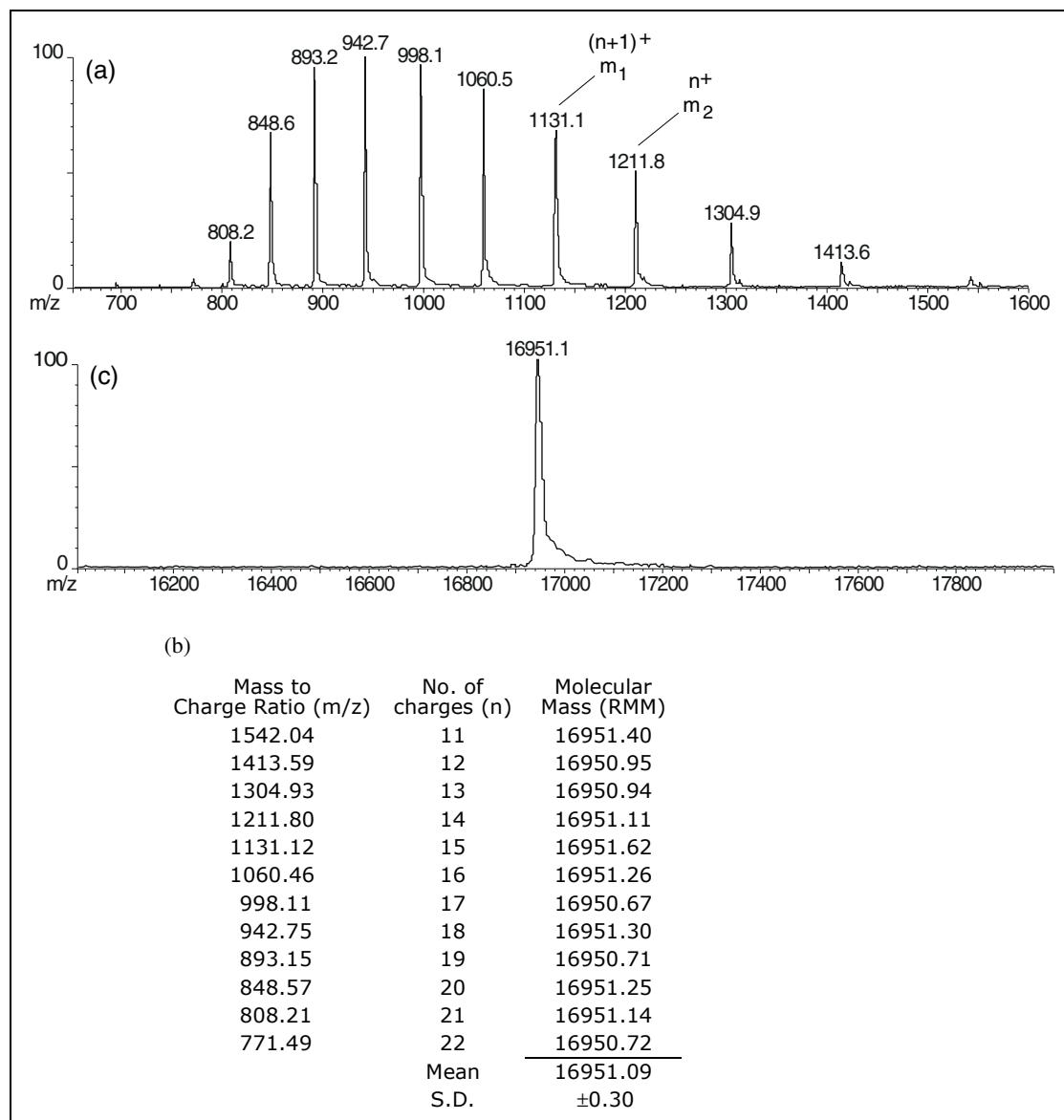


Figure 5 A sample of the protein, horse heart myoglobin, was dissolved in acidified aqueous acetonitrile (1% formic acid in $\text{H}_2\text{O}/\text{CH}_3\text{CN}$, 1:1 v/v) at a concentration of 20 pmol/l. This sample was injected into a flow of the same solvent passing at 5 $\mu\text{l}/\text{min}$. into the electrospray source to give the mass spectrum of protonated molecular ions $[\text{M}+\text{nH}]^+$ shown in (a). The measured m/z values are given in the table (b), along with the number of protons (charges; n) associated with each. The mean relative molecular mass (RMM) is 16951.09 ± 0.3 Da. Finally, the transformed spectrum, corresponding to the true relative molecular mass is shown in (c); the observed value is close to that calculated (16951.4), an error of only 0.002%.

**Laser Desorption
Mass Spectrometry
(LDMS)**

This technique depends on the use of solid matrices (e.g., cinnamic acid derivatives) to absorb energy from a laser pulse so as to volatilize and ionize proteins premixed with the matrix. Mass analysis is achieved by a Time-of-Flight analyser which, as the name suggests, measures precisely the time taken for the ions to travel from the source through the flight tube to the detector. The heavier the ion, the longer the flight time. The spectrum generally contains a protonated $[M+H^+]$ or deprotonated $[M-H^-]$ molecular ion cluster, together with a doubly-charged and perhaps further multiply charged ions. Fragmentation, and hence sequence information, is usually absent.

In principle there is no upper limit to the mass range and proteins as large as 200 kDa have been measured using as little as 1 pmole of material, making this one of the most sensitive techniques available. However, the resolution of this technique is low compared with other mass spectrometric methods, and the ions constituting the molecular mass cluster are unresolved. Heterogeneous proteins can present a problem, as the mass accuracy of 0.1% (e.g. 50 Da at 50 kDa) means that some post-translational modifications go undetected and mass changes associated with a single amino acid substitution would be unnoticed.

Electrospray (ES)

This second development has radically increased the use of mass spectrometry in biotechnology by providing an ionization technique capable of analysing large polar, thermally-sensitive biomolecules with unprecedented mass accuracy and good sensitivity.

In ES, the sample, in solution, is passed through a narrow capillary and reaches an atmospheric pressure ionization source as a liquid spray. The voltage at the end of the capillary is significantly higher (3 kV) than that of the mass analyser and so the sample emerging is dispersed into an aerosol of highly charged droplets known as the electrospray. Evaporation of solvent, aided by a stream of nitrogen, causes a decrease in the size of the droplets until eventually multiply-charged ions from individual protein molecules, free from solvent, are released and can be mass analysed.

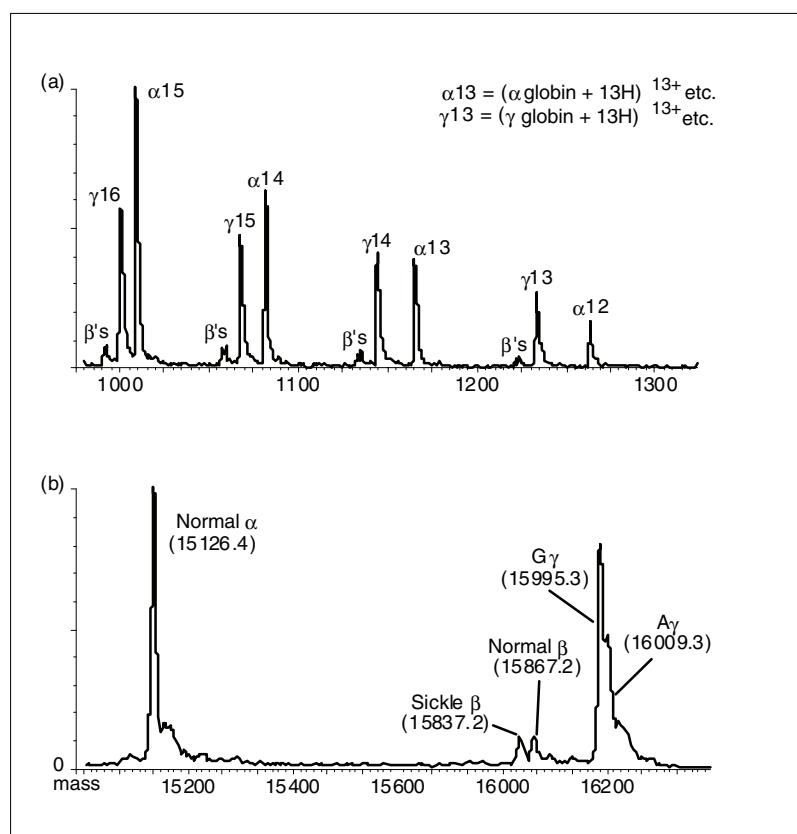


Figure 6 (a) Part of an electrospray spectrum of globins from the cord blood of a sickle cell carrier baby and, (b) the same after computer transformation onto a true molecular mass scale. Normal α , β and γ globins are clearly visible, along with the sickle cell variant, β . The two γ -globins (A , G), although having a mass difference of only 14 Da in 16,000 Da, are easily distinguished from each other.

The ions so produced are separated by their mass-to-charge (m/z) ratios. For peptides and proteins, the intact molecules become protonated with a number (n) of protons (H^+). Thus, in place of the true molecular mass (M), molecular ions have a mass of $[M+nH]$. More importantly, the ion has n positive charges resulting from addition of the n protons (H^+). Since the mass spectrometer does not measure mass directly but, rather, mass-to-charge (m/z) ratio the measured m/z value is $[M+nH]/n$. This last value is less than the true molecular mass, depending on the value of n . If the ion of true mass 20,000 Da carries 10 protons, for example, then the m/z value measured would be $(20,000+10)/10 = 2001$.

This last m/z value is easy to measure accurately and, if its relationship to the true mass is known ($n = 10$), this means the true mass can be measured very accurately also. The multiply-charged ions have typical m/z values of <3000 Da, which means that conventional quadrupole or magnetic sector analysers can be used for mass measurement. Actually, the spectrum consists of a series of multiply-charged protonated molecular ions $[M+nH]^{n+}$ for each component present in the sample. Each ion in the series differs by plus and minus one charge from adjacent ions ($[M+nH]^{n+}$; $n =$ an integer series for example, 1,2,3.... etc). Mathematical transformation of the spectrum produces a true molecular mass profile of the sample (Figure 5).

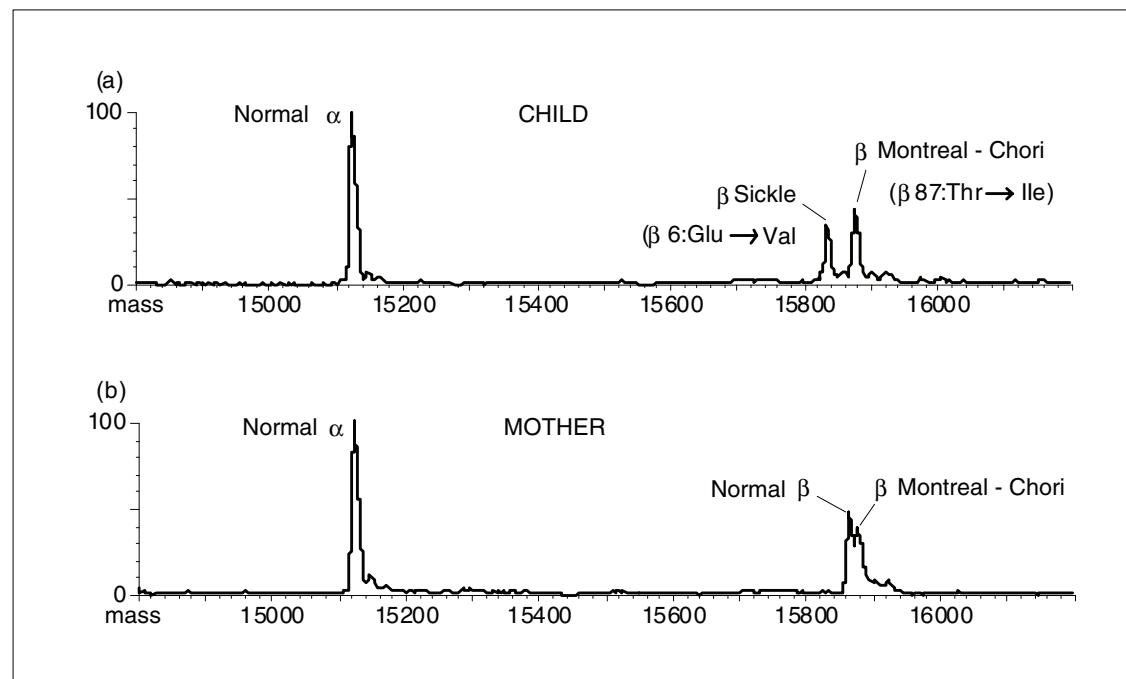


Figure 7 Electrospray mass spectra of globins from the blood of (a), a child diagnosed as having the sickle cell anaemia trait and (b) its mother. As well as the usual β -globin sickle cell variant at m/z 15837.2, a new variant (β -Montreal-Chori) appears at m/z 15879.3 and is observed in both the child and the mother.

Proteins of Large Molecular Mass

Whilst electrospray can be and is used for molecules of all molecular masses, it has had an especially marked impact on the measurement of accurate molecular mass for proteins. Traditionally, direct molecular mass measurement on proteins has been difficult, with values being obtained which were accurate to only tens or even hundreds of Daltons. The advent of electrospray now means that molecular masses of 20,000 Da and upwards can be measured with unprecedented accuracy (Figure 6). This level of accuracy means that it is also possible to identify post-translational modifications of proteins (e.g. glycosylation, acetylation, methylation, hydroxylation, etc.) and to detect mass changes associated with substitution or deletion of a single amino acid.

A typical electrospray analysis can be completed in 15 minutes with as little as 1 pmole of protein. An analysis of the globins from the cord blood of a baby (Figure 6) showed quite clearly that five globins were present, viz., the normal ones (α , β , $G\gamma$ and $A\gamma$) and a sickle cell variant (β). The last one is easily revealed in the mass spectrum even at a level of only 4% in the blood analysed.

Whilst this example shows that small differences in large masses are easily discerned by electrospray methodology, it should be noted that absolute accuracy of mass measurement is unprecedentedly high. The accuracy is sufficiently high that substitution of one amino acid by another can be detected with ease. Figure 7 illustrates this following the discovery of a new variant of β -globin, called Hb Montreal-Chori, in which a threonine residue has been substituted by isoleucine at position-87 in the β -chain. The substitution caused a mass change from 15837.2 Da to 15879.3 Da.

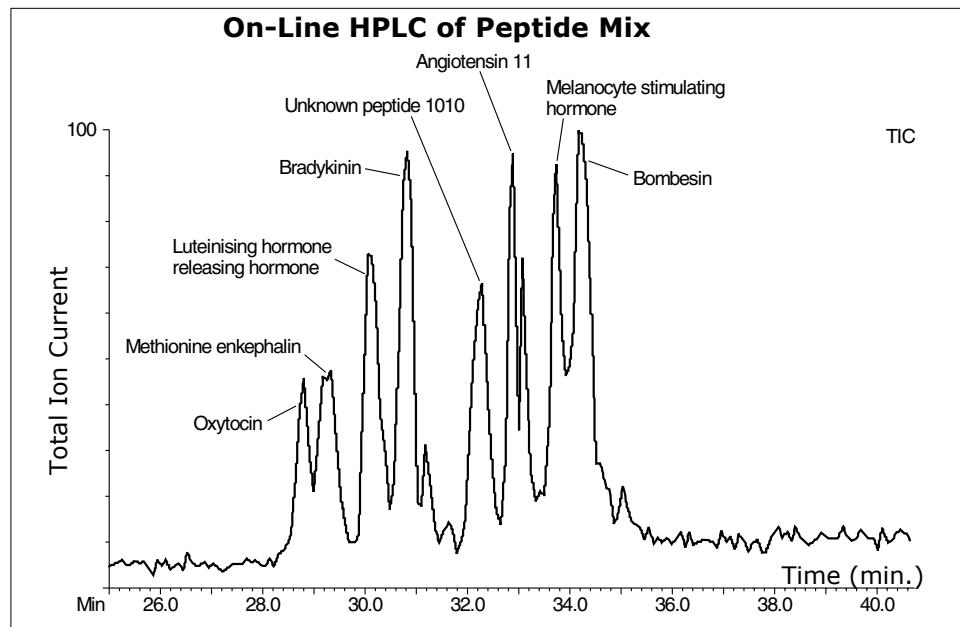


Figure 8(a) A mixture of peptides was separated by LC and the eluent was passed into an electrospray source. The total ion current trace (a) reveals the individual component peptides, each of which was identified through its measured accurate mass, an illustration of which is shown in (b) for luteinising hormone releasing hormone.

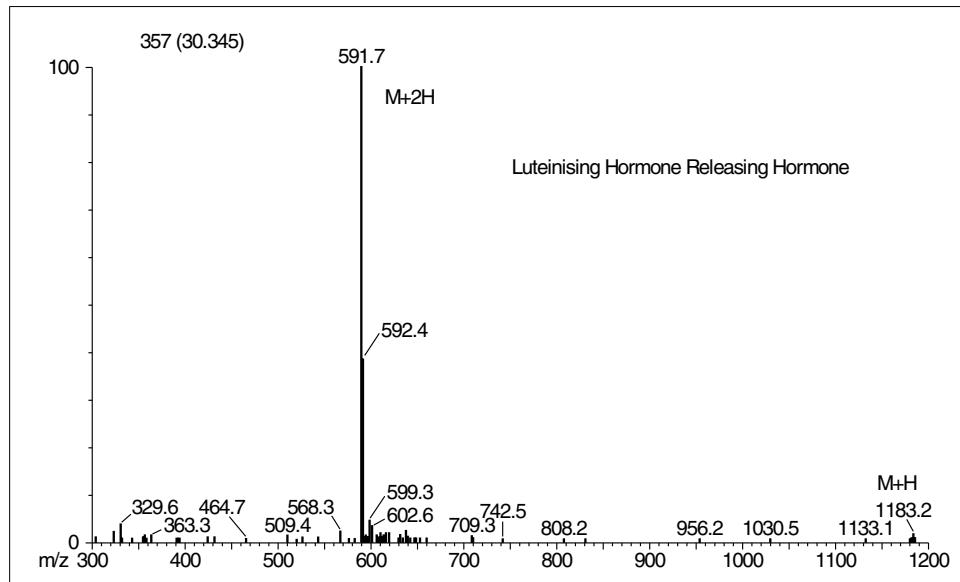


Figure 8(b)

Analysis of Peptide Mixtures

The electrospray source can be coupled directly to a liquid chromatographic column so that, as components of a mixture emerge from the column, they are passed through the source to give accurate mass data. As an example, a mixture of the peptides shown in Figure 8(a) was separated by LC and accurately mass analysed by ES. Figure 8(b) is the mass spectrum of one of these peptides (luteinising hormone releasing hormone) which gave an abundant ion, representing a doubly-protonated molecule $[M+2H]^+$ at m/z 592.4 and therefore, a true relative molecular mass (M) of 1182.8.

Conclusion

Intact peptides and proteins can be examined by a variety of new techniques, including MS-MS, dynamic-FAB and electrospray. Large masses of tens of thousands of Daltons can be accurately measured with unprecedented accuracy by electrospray.

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Back to Basics Section G: Applications

CHAPTER G7

SAMPLE INLETS FOR PLASMA TORCHES PART A: GASES

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Quick Guide

- For identification of elements present in a sample and for measurement of isotope ratios using an argon plasma torch, the sample must be introduced into the centre of the plasma flame through an inlet tube.
- Once inside the hot plasma, which is at a temperature of about 8000 K and contains large numbers of energetic electrons and ions, the samples are broken down into their constituent elements, which appear as ions. The ions are transported into a mass analyser as for example a quadrupole or a time-of-flight instrument.
- The m/z values of the ions provide identification of the elements present and the abundances of the ions gives accurate isotope ratios.
- For substances that are gases or are very volatile at ambient temperatures, it is relatively easy to introduce them into the flame. Liquids and solids are more difficult to introduce and are discussed in Parts B,C of this guide.
- The gas or vapour to be examined is mixed with argon gas to make up the needed flow of gas into the plasma flame and the sample vapour or gas is swept along with this argon make-up gas.
- If samples are introduced batchwise then the sample enters the flame as a plug and the elements are measured transiently. If the samples are introduced continuously then the measurement of isotope ratios can also be continuous as long as sample is flowing into the flame.
- There may be problems of instability in the flame if too large an amount of sample is introduced or if the sample contains substances that can interfere with the basic operation of the plasma. For example, water vapour, air and hydrogen all lead to instability if their concentrations are too high.
- In some instances, the plasma flame can go out altogether if the amounts of sample or other contaminants rise too high. This has led to the development of a wide variety of gas/liquid separators prior to introduction of a sample into the flame.

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- In some inlet devices, the volatile sample materials are first separated from entrained hydrogen gas or air by condensing them in a coolant bath. Subsequently, when all of the volatile sample components have been condensed out and the hydrogen has been swept away, the sample is reheated and is sent to the plasma flame.
- Instability in the flame leads to varying efficiencies in ion formation within the plasma (varying plasma temperature) and therefore to variations in measured isotope ratios (lack of accuracy).
- Some elements (S, Se, Te, P, As, Sb, Bi, Ge, Sn, Pb) are conveniently converted into their volatile hydrides before being passed into the plasma. The formation of the hydrides by use of sodium tetrahydroborate (sodium borohydride) may be batchwise or continuous.
- The effluent from a GC column is already in the gas phase and needs only to be mixed with argon make-up gas before passage into the flame. Precautions need to be taken to temporarily divert the GC flow when the first “solvent peak” emerges because it contains far too much material for the plasma to withstand.
- Other volatile compounds of elements may be useful for transporting samples into the plasma flame. For example, hydride reduction of mercury compounds gives the element (Hg), which is itself very volatile. Osmium may be oxidised to its volatile tetroxide (OsO_4) and some elements may be measured as their volatile acetylacetone (acac) derivatives, as with $\text{Zn}(\text{acac})_2$.

Summary

Gases and volatile materials may be swept into the centre of an argon plasma flame, where they are fragmented into ions of their constituent elements. The m/z values of ions give important information for identification of the elemental composition of a sample and precise measurement of ion abundances is used to provide accurate isotope ratios.

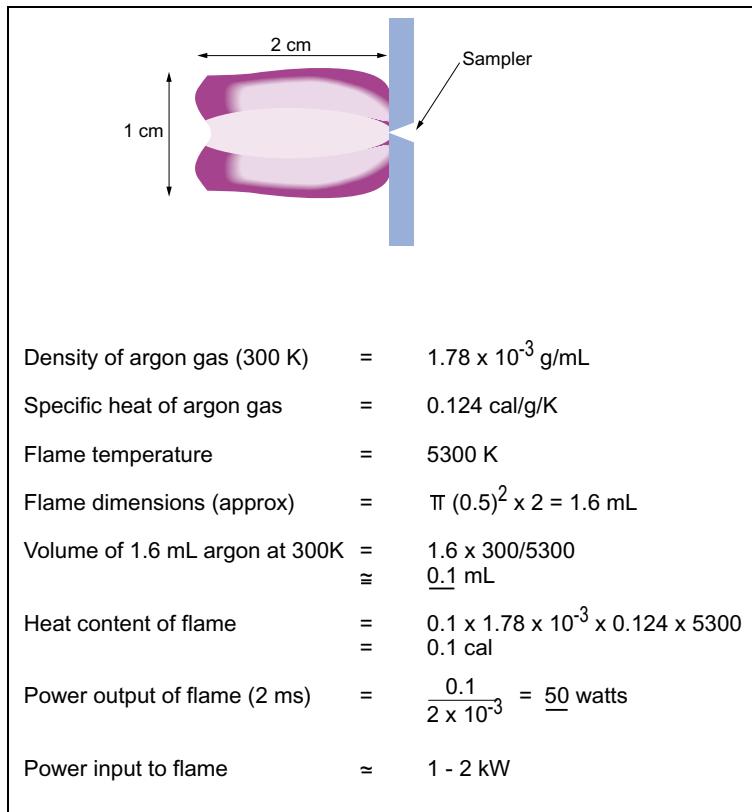


Figure 1. A plasma flame commonly has a diameter of about 1 cm and a length of about 2-3 cm. If this is regarded as being approximately cylindrical, the volume of the flame at about 5300 K is 1.6 mL and at 300 K is 0.1 mL. With a specific heat for argon of 0.124 cal/g/K and a density of 1.78×10^{-3} g/mL (at 300 K), the heat content of the flame is 0.1 cal. However, since gas flow through the hot flame occurs in a period of about 2 ms, the power output of the flame is about 50 watts. This should be compared with a power input from the high frequency electromagnetic field of about 1 kW. The seeming inconsistency between the high temperature and the low heat content arises because of the low number density of "hot" particles (the concentration of electrons and other particles in the hot flame is approximately 10^{-8} M).

SAMPLE INLETS FOR PLASMA TORCHES PART A: GASES

Introduction To examine a sample by ICPMS or ICPAES, it must be transported into the flame of a plasma torch. Once in the flame, sample molecules are literally ripped apart to form ions of their constituent elements. This fragmentation and the ionisation process are described in the Back-to-Basics guides, *Plasma Torches and Coronas*, *Plasmas and Arcs*. To introduce samples into the centre of the (plasma) flame, they must be transported there as gases or finely dispersed droplets of a solution or as fine particulate matter. The various methods of sample introduction are described in the present guides in three parts (A,B,C) to cover gases, solutions and solids. Some of these sample inlets can be multipurpose and can be used with, for example, gases and liquids but others have been designed specifically for only one kind of analysis. However, the principles governing the operation of inlet systems fall into a small number of categories, which are described in these guides. This Part A deals specifically with substances, which are normally gases or very volatile liquids at ambient temperatures.

Problems of sample introduction

The two major difficulties facing the analyst/mass spectrometrist concern firstly how to get the whole of the sample into the plasma flame efficiently and secondly how to do so without actually putting out the flame or causing instability in its performance. Although plasma flames operate at temperatures of 6000-8000 K, the mass of "gas" in the flame is very small and its thermal capacity is correspondingly small (Figure 1). This means that, if a large quantity of sample is introduced into the flame over a short period of time, the flame temperature will fall and the basic processes leading to the formation and operation of the plasma itself are also interfered with. Therefore introduction of samples into a plasma flame needs to be controlled and hence there is a need for special sample introduction techniques to deal with different kinds of samples. A major problem with introducing material other than argon into the plasma flame is that the additives may interfere with the process of electron formation, a basic factor in keeping the flame selfsustaining. If electrons are removed from the plasma by secondary processes faster than they can be replaced by the primary generation process then the plasma process ceases, viz., the flame will go out. Fluctuations in flame temperature and performance lead to significant accompanying variations in sample ion yield, often over short periods of time and these fluctuations affect accurate measurement of isotope ratios. Thus, sample preparation and manipulation are important and,

Table. Volatile hydrides useful for ICPMS determination of elemental compositions and isotope ratios

Arsenic	AsH ₃
Antimony	SbH ₃
Bismuth	BiH ₃
Germanium	GeH ₄
Lead	PbH ₂
Mercury	Hg ^a
Phosphorus	PH ₃ ^b
Selenium	SeH ₂
Sulphur	SH ₂
Tellurium	TeH ₂
Tin	SnH ₂

^a Mercury does not form a hydride but any mercury compounds present are reduced to the element itself, which being a volatile liquid is carried along into the plasma flame.

^b This hydride is formed from phosphates and similar anions of phosphorus only on heating the phosphate to 500 °C with NaBH₄ in the dry state.

Figure 2. A number of elements form volatile hydrides, as shown in the Table. Some elements form very unstable hydrides and these have too transient an existence to exist long enough for analysis. Many elements do not form stable hydrides or do not form them at all. Some elements such as sodium or calcium form stable but very involatile solid hydrides. The volatile hydrides listed in the Table are gaseous and sufficiently stable to allow analysis, particularly as the hydrides are swept into the plasma flame within a few seconds of being produced. In the flame, the hydrides are decomposed into ions of their constituent elements.

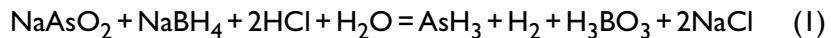
for any one type of inlet system, judicious choice of inlet conditions and sample preparation by the operator of the instrument can avoid the worst aspects of the problems just described.

Introduction of gases

Fundamentally, introduction of a gaseous sample is the easiest option for ICP-MS because all of the sample can be passed efficiently along the inlet tube and into the centre of the flame. Unfortunately, gases are mainly confined to small molecular mass compounds and many of the samples that need to be examined cannot be vaporised easily. Nevertheless, there are some key analyses, which are carried out in this fashion, the major one being the generation of volatile hydrides. Other methods for volatiles are dealt with below. An important method of analysis uses lasers to vaporise "involatile" samples such as bone or ceramics. With a laser, ablated (vaporized) sample material is swept into the plasma flame before it can condense out again. Similarly, electrically heated filaments or ovens are also used to volatilise solids, the vapour of which is then swept by argon make-up gas into the plasma torch. However, for convenience, such methods of sample introduction for solids are discussed fully in Part C of this series.

(a) Analysis of elemental hydrides (MH_n)

The elements listed in the table of figure 2 are of importance as environmental contaminants and their analysis in soils, water, seawater, foodstuffs and for forensic purposes is carried out routinely. For these reasons, methods have been sought to analyse samples of these elements quickly and easily without significant pre-preparation. One way to unlock these elements from their compounds or salts, in which form they are usually found, is to reduce them to their volatile hydrides through the use of acid and sodium tetrahydroborate (sodium borohydride), as shown in equation (1) for sodium arsenite.



The volatile hydride (arsine in equation 1) is swept by a stream of argon gas into the inlet of the plasma torch. The plasma flame decomposes the hydride to give elemental ions. For example, arsine gives arsenic ions at m/z 75. The other elements listed in figure 2 also yield volatile hydrides, except for mercury salts, which are reduced to the element (Hg), which is volatile. In the plasma flame, the arsine of equation (1) is transformed into As^+ ions. The other elements of figure 2 are converted similarly into their elemental ions.

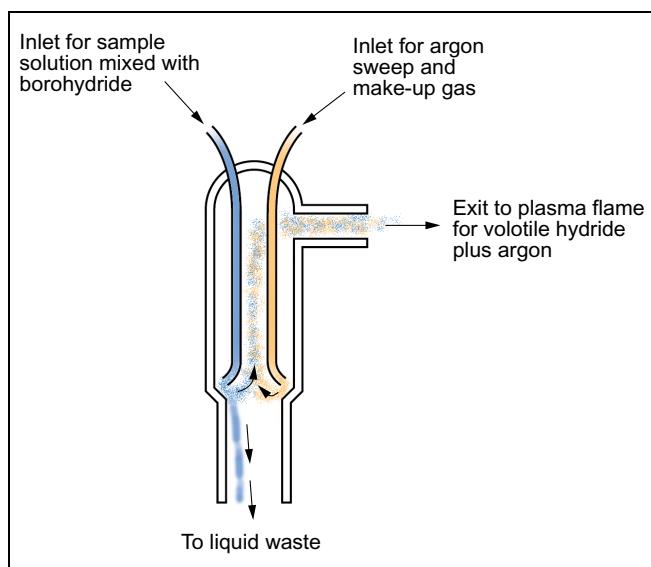


Figure 3. A schematic illustration of a typical inlet apparatus for separating volatile hydrides from the analyte solution, in which they are generated on reduction with sodium tetrahydroborate. When the mixed analyte solution containing volatile hydrides enters the main part of the gas/liquid separator, the volatiles are released and mix with argon sweep and make-up gas, with which they are transported to the centre of the plasma. The unwanted analyte solution drains from the end of the gas/liquid separator. The actual construction details of these gas/liquid separators can vary considerably but all serve the same purpose. In some of them, there can be an intermediate stage for removal of air and hydrogen from the hydrides before the latter are sent to the plasma.

A major advantage of this hydride approach lies in the separation of the remaining elements of the analyte solution from the element to be determined. Because the volatile hydrides are swept out of the analyte solution, the latter can be simply diverted to waste and not sent through the plasma flame itself. This means that potential interferences from sample preparation constituents and by-products are reduced to very low levels. For example, a major interference for arsenic analysis arises from ions ArCl^+ having m/z 75,77, which have the same integral m/z value as that of As^+ ions themselves. Thus, any chlorides in the analyte solution (for example, from sea water) could produce serious interference in the accurate analysis of arsenic. The option of diverting the used analyte solution away from the plasma flame is beneficial to accurate, sensitive analysis of isotope concentrations. Inlet systems for generation of volatile hydrides may operate continuously or batchwise.

Unfortunately, the borohydride method of forming volatile hydrides also produces hydrogen gas as a by-product (equation 1) and this can interfere with the performance of the plasma flame. In sufficiently large concentrations, hydrogen can even cause the flame to go out because it has a high ionisation potential, which interferes with the primary cascade production of electrons necessary to maintain the plasma (see Back-to-Basics, Coronas, Plasmas and Arcs and Plasma Torches). Other gases produced as by-products to the formation of volatile hydrides are CO_2 and H_2O and these can also lead to instability in the plasma if present in too large a concentration. Devices for separating unused analyte or hydrogen have been produced for both batchwise and continuous use. One such separator is illustrated in figure 3. Hydrogen and entrained air can be removed from the gas stream going into the plasma by freezing out the volatile hydrides in a trap while diverting the hydrogen out of the system; the sample hydrides are released by warming the condensate and sweeping them to the flame with argon as they volatilize. Alternatively, hydrogen may be removed by passing the newly produced hydride gases through a tubular membrane separator, which allows easy diffusion of the small hydrogen molecules through its walls but not those of the much larger hydrides, which pass along the tube and into the plasma flame.

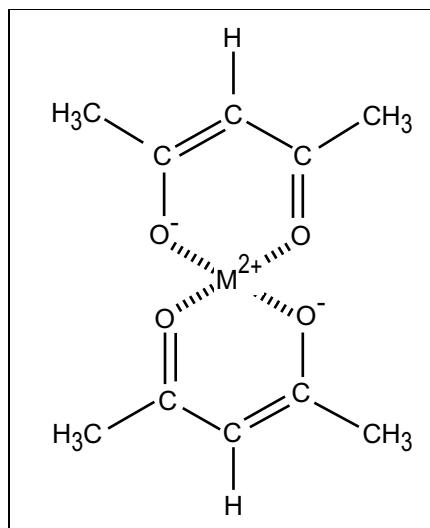


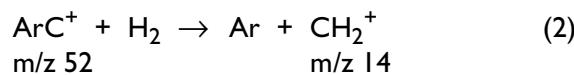
Figure 4. The chemical structure of a typical divalent metal acetylacetonate, for which the abbreviation would be $\text{M}(\text{acac})_2$. These compounds are "internally" bonded ionically and complexed to oxygen at the same time. Thus, their intramolecular forces are very strong (they are stable) but their intermolecular forces are weak (they are volatile).

(b) Other volatile materials produced chemically

Chemical ingenuity in using the properties of the elements and their compounds has allowed analyses to be carried out by processes analogous to the generation of hydrides. Osmium tetroxide is very volatile and can be formed easily by oxidation of osmium compounds. Some metals form volatile acetylacetones (acac), as with iron, zinc, cobalt, chromium and manganese (Figure 4). Iodides may be oxidised easily to iodine (another volatile element in itself) and carbonates or bicarbonates can be examined as CO_2 after reaction with acid.

(c) Introduction of gases directly

This is probably the easiest method of introduction. A gas sample can be introduced through the inlet into the centre of the plasma flame, usually after first mixing the sample with argon to dilute it and carry it forward (the argon is then known as a make-up gas). For example, semiconductor grade gases such as silane (SiH_4) can be analysed by ICP-MS by mixing the gas with argon and admitting it into the plasma flame in small defined amounts (microlitres) through use of a gas sampling loop. Where organic compounds are used, it is often beneficial to add low concentrations of oxygen or air to help burn them. Nitrogen or xenon can be used to reduce interferences caused by the formation of carbides. An example of the latter is an interference in chromium determination at m/z 52 caused by formation in the plasma of the carbide, ArC^+ (also at m/z 52). In one commercial instrument, this sort of interference is dealt with more efficiently through use of a hexapole collision chamber sited after extraction of the ions from the plasma but before passage of the ions into the mass analyser proper for measurement of m/z values. This collision chamber, containing a low pressure of hydrogen as the collision gas, causes the decomposition of the carbide species through ion/molecule reactions, as shown in equation (2).



(d) Introduction of gases and vapours by coupling to a gas chromatograph (GC-ICP-MS)

Ostensibly, a GC apparatus should be ideal as an inlet for a plasma torch because the effluents from the chromatographic column are already in the gas phase and can be passed straight into the plasma flame. However, most analyses carried out by GC involve carbon compounds, with oxygen, nitrogen and the halogens as commonly occurring constituents. The gas flow from the capillary GC column is normally increased (made up) by mixing in more argon gas with the effluent before it reaches the flame. Introduction of a sample into a GC column as a solution means there is usually a large "solvent peak" at the start of any gas chromatogram. Because the eluting solvent contains a relatively large amount of material, it must be diverted from the flame else, otherwise, it would make the plasma very

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unstable or even put it out completely. Therefore, all GC connections to an ICP-MS instrument contain a diverter to switch the gas flow from the flame during the few seconds required for the solvent to elute from the end of the GC column.

Gas chromatography is mostly used for the analysis of carbon-containing compounds, which may or may not contain oxygen, nitrogen, sulphur or halogens as the principal co-elements. If ICP-MS is simply used as a detector for GC effluents, without any other purpose, it becomes a very expensive coupled device compared with GC using a simple flame ionisation detector. Therefore, to couple GC with an ICP-MS instrument, there must be factors present, which are so attractive as to override the cost disadvantages. Element ratio (isotope) analyses and multiple identification of elements are major reasons for using GC-ICP-MS. Where mixtures of volatile compounds must be separated and examined for their element compositions and ratios then GC-ICP-MS becomes an important on-line technique. Such conjoint favourable economic circumstances are not too common and probably mostly for this reason, GC-ICP-MS is not so widely used as might be imagined, given its attractions. However, many applications of its use or potential use have been described in research papers and it continues to be developed. For example, organo lead, tin and mercury compounds have been separated and analysed on-line by GC-ICP-MS.

(e) Other vapour introduction systems

These are discussed in Parts B,C because, although ultimately liquids and solids are introduced to the plasma flame as vapours, these samples are usually prepared differently from naturally gaseous ones. For example, electrothermal (oven) or laser heating of solids and liquids to form vapours is used extensively to get the samples into the plasma flame. At one extreme with very volatile liquids, no heating is necessary but at the other, very high temperatures are needed to vaporize a sample. For convenience, the electrothermal and laser devices are discussed in Part C rather than here.

Summary

Gases and vapours of volatile liquids may be introduced directly into a plasma flame for elemental analysis or for isotope ratio measurements. Some elements may be examined by first converting them chemically into volatile forms, as with the formation of hydrides of arsenic, and tellurium. It is important that not too much analyte should pass into the flame as the extra material introduced into the plasma may cause it to become unstable or even to go out altogether, thereby compromising accuracy or continuity of measurement

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Back to Basics Section G: Applications

CHAPTER G7-1

SAMPLE INLETS FOR PLASMA TORCHES PART B: LIQUID INLETS

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Quick Guide

- For identification of elements present in a sample and for measurement of isotope ratios using an argon plasma torch, the sample must be introduced into the centre of the plasma flame through an inlet tube.
- Once inside the hot plasma, which is at a temperature of about 8000 K and contains large numbers of energetic electrons and ions, the samples are broken down into their constituent elements, which appear as ions. The ions are transported into a mass analyser as for example a quadrupole or a time-of-flight instrument.
- The m/z values of the ions provide identification of the elements present and the abundances of the ions gives accurate isotope ratios.
- For substances that are gases or are very volatile at ambient temperatures, it is relatively easy to introduce them into the flame; gases and vapours are discussed in Part A. Solids are more difficult to handle and are discussed in Part C of this guide.
- A liquid sample must be vaporised to a gas or, more likely to a vapour consisting of an aerosol of gas, small droplets and even small particles of solid matter. To be examined, the aerosol is mixed with argon gas to make up the needed flow of gas into the plasma and is then swept into the flame.
- If samples are introduced batchwise then each one enters the flame as a plug and the elements are measured transiently and, if more than one m/z ration must be examined, the analyser needs to be a quadrupole or time-of-flight instrument.
- If samples are introduced continuously then the measurement of isotope ratios can also be continuous as long as sample is flowing into the flame and several m/z ratios can be examined with almost any kind of mass spectrometer.
- There may be problems of instability in the flame if too large an amount of vaporised liquid is introduced or if the sample contains substances that can interfere with the basic operation of the plasma. For example, water vapour, organic solvents, air and hydrogen in too large a concentration all lead to instability in the plasma flame if their concentrations become too high.

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- Instability in the flame leads to varying efficiencies in ion formation within the plasma (varying plasma temperature) and therefore to variations in measured isotope ratios (lack of accuracy).
- In some instances, the plasma flame may go out altogether if the levels of sample or other contaminants rise too high. This has led to the development of a wide variety of gas/liquid separators and/or desolvation chambers prior to introduction of a sample into the flame. These reduce the amount of solvent flowing into the flame.
- Some elements (S, Se, Te, P, As, Sb, Bi, Ge, Sn, Pb) in liquid samples are conveniently converted into their volatile hydrides before being passed into the plasma, as discussed in Part A. For some samples, any volatile solvent is first evaporated in a sample holder, which is then heated strongly to vaporise the resulting solid residue (described in Part C).

Summary

Solutions may be examined by ICP-MS by either evaporating the solvent first and then volatilising the solid residue or by nebulizing the solution and desolvating the resulting spray of fine droplets. After vaporisation, residual sample (solute) constituents are swept into the centre of an argon plasma flame, where they are fragmented into ions of their constituent elements. The m/z values of ions give important information for identification of the elemental composition of the sample and measurement of ion abundances is used to provide accurate isotope ratios.

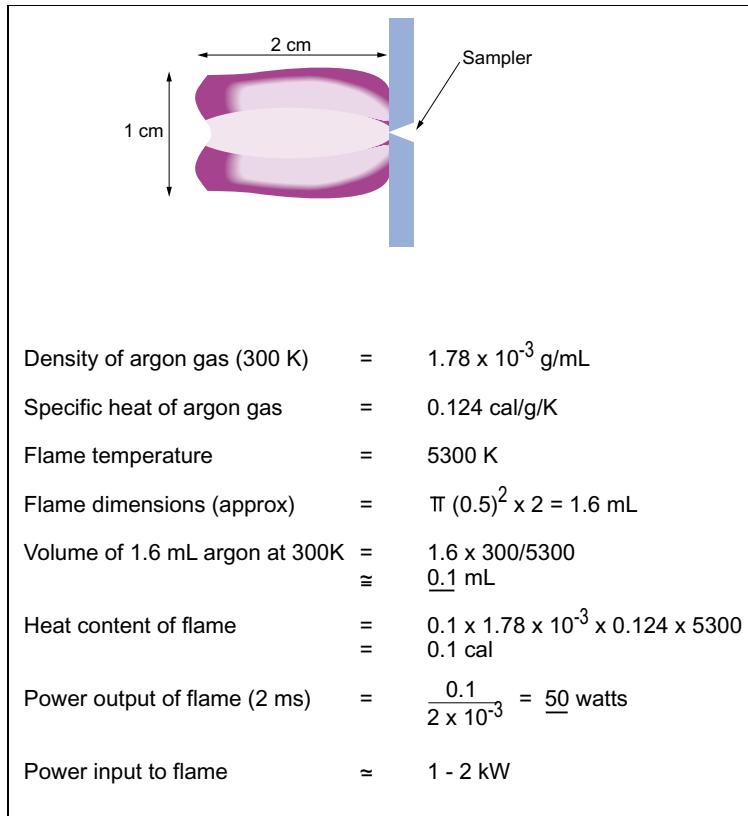


Figure 1. A plasma flame commonly has a diameter of about 1 cm and a length of about 2-3 cm. If this is regarded as being approximately cylindrical, the volume of the flame at about 5300 K is 1.6 mL and at 300 K is 0.1 mL. With a specific heat for argon of 0.124 cal/g/K and a density of 1.78×10^{-3} g/mL (at 300 K), the heat content of the flame is 0.1 cal. However, since gas flow through the hot flame occurs in a period of about 2 ms, the power output of the flame is about 50 watts. This should be compared with a power input from the high frequency electromagnetic field of about 1 kW. The seeming inconsistency between the high temperature and the low heat content arises because of the low number density of "hot" particles (the concentration of electrons and other particles in the hot flame is approximately 10^{-8} M).

SAMPLE INLETS FOR PLASMA TORCHES PART B: LIQUID INLETS.

Introduction To examine a sample by ICPMS or ICPAES, it must be transported into the flame of a plasma torch. Once in the flame, sample molecules are literally ripped apart to form ions of their constituent elements. These fragmentation and the ionisation processes are described in the Back-to-Basics guides, *Plasma Torches and Coronas*, *Plasmas and Arcs*. To introduce samples into the centre of the (plasma) flame, they must be transported there as gases or finely dispersed droplets of a solution or as fine particulate matter. The various methods of sample introduction are described in the present guides in three parts (A,B,C) to cover gases, solutions (liquids) and solids. Some of these sample inlets can be multipurpose and can be used with, for example, gases and liquids but others have been designed specifically for only one kind of analysis. However, the principles governing the operation of inlet systems fall into a small number of categories, which are described in these guides. This Part B deals specifically with substances, which are normally liquids at ambient temperatures. This sort of inlet is the commonest in analytical work.

Problems of sample introduction

The two major difficulties facing the analyst/mass spectrometrist concern firstly how to get the whole of the sample into the plasma flame efficiently and secondly how to do so without causing the flame to go out or causing instability in its performance. Although plasma flames operate at temperatures of 6000-8000 K, the mass of "gas" in the flame is very small and its thermal capacity is correspondingly small (Figure 1). This means that, if a large quantity of sample is introduced into the flame over a short period of time, the flame temperature will fall and the basic processes leading to the formation and operation of the plasma itself are also interfered with. Therefore introduction of samples into a plasma flame needs to be controlled and hence there is a need for special sample introduction techniques to deal with different kinds of samples. A major problem with introducing material other than argon into the plasma flame is that such additives may interfere with the process of electron formation, a basic factor in keeping the flame selfsustaining. If electrons are removed from the plasma by secondary processes faster than they can be replaced by the primary generation process then the plasma process ceases, viz., the flame goes out. Fluctuations in flame temperature and performance lead to significant accompanying variations in sample ion yield, often over short periods of time and these fluctuations affect accurate measurement of isotope ratios. Thus, sample preparation and manipulation are important and, for any

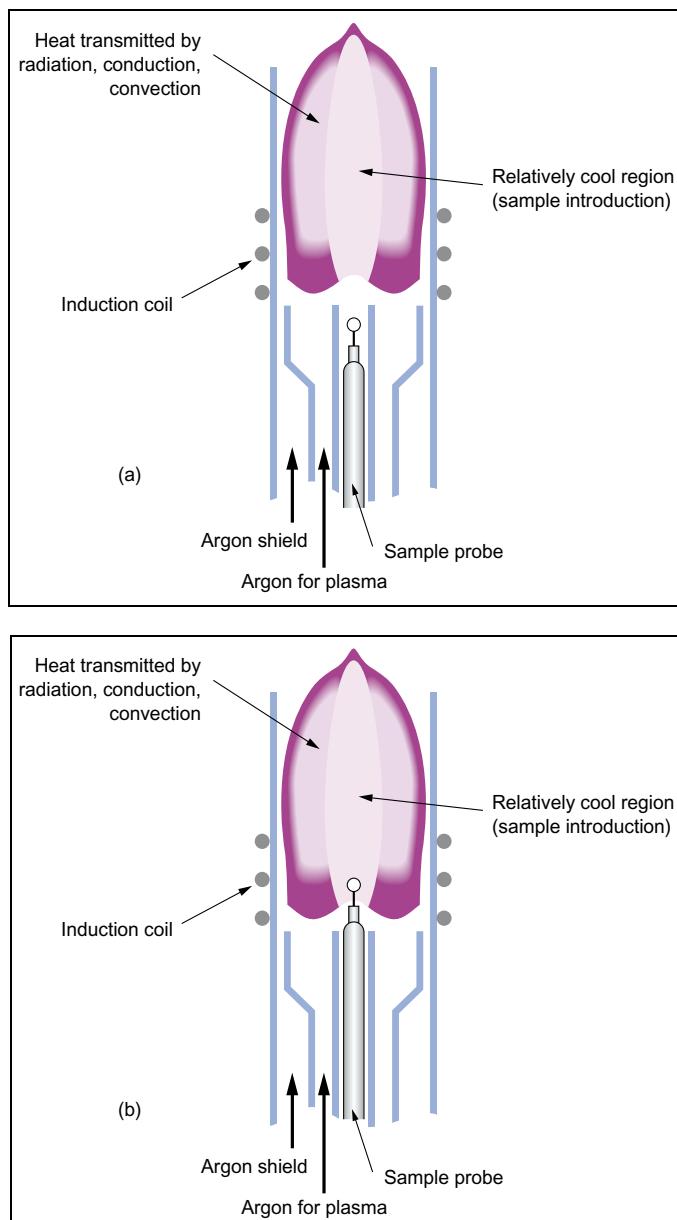


Figure 2. The diagram indicates the method of direct insertion of a sample into the plasma flame. Initially, the sample, held on a wire or in a small graphite or metal "boat", is placed just below the flame until conditions have stabilised. The probe is then moved up so that the sample holder enters the flame, where ablation occurs and the sample is simultaneously fragmented and ionised. The plasma flame is shown vertical instead of the usual horizontal so that the sample cannot run out or drop out of any cup that might be used for sample insertion. If there is no problem of containing or holding the sample then the flame can be in its more normal horizontal alignment.

one type of inlet system, judicious choice of inlet conditions and sample preparation by the operator of the instrument can avoid the worst aspects of the problems just described.

Liquid inlets

Suitable inlets commonly used for liquids or solutions may be separated into three major classes, two of which are discussed in Parts A,C. The commonest method for introduction of solutions uses the nebulizer/desolvation inlet discussed here. For greater detail on types and operation of nebulizers, the Back-to-Basics guide, *Nebulizers* should be consulted. It might be noted that, for all samples that have been previously dissolved in a liquid (dissolution of sample in acid or alkali or solvent), it is important that high purity liquids be used if cross-contamination of sample is to be avoided. Once the liquid has been vaporised prior to introduction of residual sample into the plasma flame, any non-volatile impurities in the liquid will have been mixed with the sample itself and these impurities will appear in the results of analysis. The problem may be partially circumvented by use of "blanks", viz., the separate examination of levels of residues left by solvents in the absence of any sample.

Direct insertion methods (Direct solids insertion, DSI)

This topic has been discussed in Part C since the approach usually requires an initial evaporation of solvent from a solution by moderate heating in a gas stream so as to leave behind the solute (the analytical sample). The resulting residual sample is then heated strongly so as to vaporise it. Typically, a solution is placed onto a heat resistant wire or onto a graphite probe and then the solvent is allowed to evaporate or is encouraged to do so by application of heat, directly or indirectly. The residual solid on its metal or graphite support is placed just below the a plasma flame, which is allowed to stabilise for a short time. The probe and sample are then driven into the high temperature flame, which causes vaporisation, fragmentation and ionisation (Figure 2). Because the heat capacity of the flame is relatively small, the sample holder and sample should have as low a thermal mass as possible so as not to interfere with the operation of the flame. With the direct insertion method, samples appear transiently in the flame and therefore, if a wide range of elements is to be examined, the mass spectrometer should be one which can span a wide m/z range in a short space of time the sample takes to pass through the flame (quadrupole, time-of-flight). Further details of the DSI technique are shown in Part C.

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**Electrothermal heating methods
(Electrothermal vaporisation, ETV)**

This topic has been described in part C because application of heat to remove the solvent from a solution results in a residual solid (or other relatively involatile material), which must then be heated strongly to effect vaporisation to form an aerosol. The latter is swept into the plasma flame with a flow of argon gas. To examine a solution by the ETV method, it is first placed on a wire or into a "boat" or "cup" or in an "oven" and the solvent is evaporated by application of moderate heat. The resulting solvent vapours are routed away from the plasma flame or the evaporation is done slowly so as not to put out the flame altogether. After evaporation of the solvent, the flame is allowed to stabilise if necessary and then the sample holder (probe) is heated strongly by electrical means so as to vaporise the residual solid sample as an aerosol, the droplets of which are led into the flame by argon gas flowing through a transfer line. In one variation of this method, the sample on its sample holder (a wire) is placed close to the flame in the sample inlet tube and the wire is heated electrically to drive off first the solvent and then the sample (this apparatus is similar to that shown in Figure 2). Further details of the ETV technique are described in Part C.

Nebulizer methods

By far the most general method of introducing a liquid sample into a plasma flame is by splitting up the liquid into a stream of droplets (nebulization), which is led along a transfer line and then into the centre of the plasma flame. During transfer, the droplets evaporate and become very small, often being only residual analyte. Once in the flame, the small drops of sample are fragmented and ionised. The process may be used in such a way as to generate a transient signal or a continuous one.

Nebulization has been known for many years and is commonly met with in hair and paint spays and similar devices. For application to sample introduction into an ICP instrument, greater control is needed. For example, if the highest sensitivities of detection are to be maintained, it is desirable that most of the sample solution should enter the flame and not be lost beforehand. The range of droplet sizes that is produced should be as small as possible and, preferably, the droplets themselves should be of the order of a few micrometres in diameter. Large droplets contain a lot of solvent which, if evaporated inside the plasma itself, leads to instability in the flame with concomitant variations in instrument sensitivity. Sometimes the flame may even be snuffed out by the amount of solvent present because of interference with the basic mechanism of flame propagation. For these reasons, nebulizers for use in ICP mass spectrometry usually combine a means of desolvating the initial spray of droplets so that they shrink to a smaller, more uniform size or sometimes even into small particles of solid matter (particulates).

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Nebulizers may be divided into several main types. The pneumatic forms work on the principle of breaking up a stream of liquid into droplets by mechanical means; the liquid stream is forced through a fine nozzle and breaks up into droplets. There may be a concentric stream of gas to aid the formation of small droplets. The liquid stream may be directed from a fine nozzle at a solid target so that, on impact, the narrow diameter stream of liquid is broken into many tiny droplets. There are variants on this approach, described in the Back-to-Basics guide, *Nebulizers*.

Thermospray nebulizers operate on the principle of converting a thin stream of liquid into a rapidly expanding vapour through application of heat. As the liquid stream to be nebulized reaches the end of a capillary tube, it meets a short region of tubing, which has been pre-heated to such a temperature that the solvent begins to boil rapidly. The resultant expanding gas stream mixes with unvapourised liquid to give a fine spray of droplets from the end of the capillary tube. These sorts of nebulizer have been attractive for introduction of solution eluants from liquid chromatography instruments, particularly those using the very narrow "nanobore" columns. Thermospray has been described (see Back-to-Basics, *Thermospray Ionisation*). Electrospray forms another mode of nebulization suitable for liquid chromatography and is discussed in detail in the Back-to-Basics guide, *Electrospray Ionisation*.

Droplets may be produced ultrasonically. Application of a rapidly oscillating electric potential (200-1000 kHz) to certain types of inorganic crystal causes a face of the crystal to oscillate at a similar rate (piezoelectric effect). For use in piezoelectric devices, the moving face of the crystal is usually protected by fastening a thin metal plate to it. Since erosive and corrosive effects are frequently common with such devices, chemically and physically robust metals such as titanium are used to provide this facing to the piezoelectric crystal. Any thin stream of liquid directed at a steep angle onto the rapidly oscillating surface of a piezoelectric crystal is subjected to a "standing" acoustic wave which breaks it down into droplets. The rate at which droplets are produced is much greater than for pneumatic nebulizers and a desolvation chamber is necessary to avoid overlarge amounts of solvent and sample entering the flame and causing instability.

Once an aerosol has been produced, it is usually in the form of vapour (gas) from the solvent plus droplets of the original solution and sometimes also particulate (solid) matter. The droplets usually cover a wide range of sizes, which are not static but vary as the droplets proceed towards the plasma flame. As the aerosol is swept towards the flame by a flow of argon gas, small droplets grow bigger by collision with others and all droplets become smaller as solvent evaporates. Thus, the initial aerosol produced at the nebulizer will

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have changed its size distribution by the time it meets the plasma flame. Most of these changes will have occurred within about 100-200 milliseconds. The changes are frequently assisted by incorporation of a desolvation chamber between the nebulizer and the flame.

Desolvation chambers

The solutions introduced into an ICP-MS instrument are commonly water-based (acids, alkalies, salts or sea water) but may also be organic based, as with effluents from liquid chromatography columns. The two types of solvent cause different problems for a plasma flame but high concentrations of either need to be avoided. A plasma flame has low thermal capacity and depends critically on a continuous formation of electrons and ions. If the flame is diluted by large amounts of vapour or by the presence of certain elements, its temperature is reduced so that ionisation efficiency for sample molecules is reduced and hence there is a reduction in ionisation efficiency. At best, a variable efficiency of ionisation causes problems with the measurement of accurate abundances of ions and, at worst, the flame may be put out altogether. Apart from these problems, inside the flame, water can give rise to interferences due to oxide formation within droplets before evaporation is complete. This effect is particularly marked for the elements vanadium, molybdenum, lanthanum, cerium, thorium and uranium. For organic solvents, flame efficiency can even be increased in that chlorinated solvents give better efficiencies than do hydrocarbons. An excess of organic solvent can lead to a build-up of carbon deposits on the sampling cone situated at the tip of the plasma flame (see Back-to-Basics, *Plasma Torches*). These factors make it necessary that the amount of solvent vapour entering the flame should be reduced to as low a level as possible and that any droplets or particulates entering the flame should also be small and of as uniform a droplet size as possible. Desolvation chambers are designed to optimise these factors so as to maintain a near constant efficiency of ionisation and to flatten out fluctuations in droplet size from the nebulizer. Droplets of less than 10 micrometres in diameter are preferred. For the flow rates of less than about 10 microlitres per minute issuing from micro- or nano-bore liquid chromatography columns, a desolvation chamber is unlikely to be needed.

The simplest desolvation chambers consist simply of a tube heated to about 150 °C through which the spray of droplets passes. During passage through this heated region, solvent evaporates rapidly from the droplets and forms vapour. The mixed vapour and residual small droplets or particulates of sample matter are swept by argon through a second cooled tube, which allows vapour to condense out on its

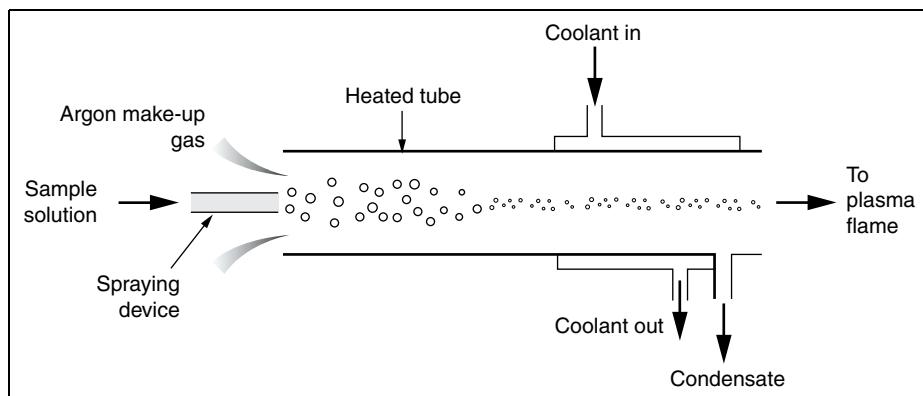


Figure 3. In a typical desolvation chamber, the initial sample solution is nebulized by some form of spraying device. The resultant aerosol, a mix of gas, vapour and droplets having a wide range of sizes is swept through a heated tube by a flow of argon gas. The tube is typically held at about 150 °C. Here, much of the solvent is vaporised from the droplets, which are greatly reduced in size to become small multimolecular aggregates (very small droplets). This mixture of small gaseous solvent molecules and the larger analyte particles or aggregates passes into a cool region, where the solvent molecules, diffusing more rapidly from the stream of gas and droplets, condense onto the walls of the tube and are run off as liquid waste. The remaining small droplets and particulates together with traces of solvent pass on into the centre of the plasma flame, where fragmentation and ionisation of sample occurs. In other devices, the desolvation chamber consists of a membrane through which the small solvent molecules can diffuse but the larger droplets and particulates cannot; these last pass on to the flame.

walls and to be run off to waste (Figure 3). This second tube may be maintained at a temperature of about 0 to -10°C. Even more elaborate successive alternate heating and cooling systems have been introduced so as to remove almost all of the solvent.

A second form of desolvation chamber relies on diffusion of small vapour molecules through pores in a Teflon membrane in preference to the much larger droplets (molecular agglomerations), which are held back. These devices have proved popular with thermospray and ultrasonic nebulizers, both of which produce large quantities of solvent and droplets in a short space of time. Bundles of heated hollow polyimide or "Nafion" fibres have been introduced as short, high surface area membranes for efficient desolvation.

Conclusion

Solutions may be examined by ICP-MS by either first removing the solvent (direct and electrothermal methods) and then vaporising residual sample solute or they are nebulized by changing any solution into a spray of droplets, which is swept into the plasma flame, after removing excess of solvent in a desolvation chamber. The direct and electrothermal methods are not as convenient as the nebulization inlets for multiple samples but the former are generally much more efficient in transferring samples into the flame for analysis.

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Back to Basics Section G: Applications

CHAPTER G7-2

SAMPLE INLETS FOR PLASMA TORCHES PART C: SOLID INLETS

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Quick Guide

- In using an argon plasma torch for identification of elements present in a sample and for measurement of isotope ratios, the sample must be introduced into the centre of the plasma flame through an inlet tube.
- Once inside the hot plasma, which is at a temperature of about 8000 K and contains large numbers of energetic electrons and ions, the sample molecules are broken down into their constituent elements, which appear as ions. The ions are transported into a mass analyser, as for example a quadrupole or a time-of-flight instrument, for measurement of m/z values and ion abundances.
- The m/z values provide identification of the elements present and the abundances of the ions give accurate isotope ratios.
- For substances that are gases or are very volatile at ambient temperatures, it is relatively easy to introduce them into the flame. Liquids and solids are more difficult and are discussed in Parts B,C of this guide.
- The solid to be examined must be vaporized in some way. This may be done by using the heat of the plasma flame or, more usually, the solid is ablated separately and the ablated aerosol is mixed with argon gas and swept into the centre of the flame.
- If solid samples are vaporized quickly then the sample enters the flame as a small plug and the elements must be measured over a short period of time. This mode is useful for high sensitivity because all of the sample passes through the flame in a short time (the abundances of ions appear as a "sharp" peak on the output. If samples are introduced continuously then ultimate sensitivity may be reduced but isotope ratios can be determined continuously to provide high accuracy.
- There may be problems of instability in the flame if too large an amount of sample is introduced or if the sample contains substances that can interfere with the basic generation of electrons and ions in the plasma. For example, water vapour, air and hydrogen all lead to instability if their concentration is too high.
- In some instances, the plasma flame can go out altogether if the levels of sample or other contaminants rise to too high a level.

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- Instability in the flame leads to varying efficiencies in ion formation within the plasma (varying plasma temperature) and therefore to variations in measured isotope ratios (lack of accuracy).
- Some solid samples may be vaporised easily but others require very high temperatures. The inlet systems need to be able to cover a vaporization range of about 100-2000 °C.
- Some solids inlet systems are suitable also for liquids (solutions) in a solution of a sample is first evaporated at low temperatures to leave a residual solid analyte, which must then be vaporised at higher temperatures.
- The major methods used for vaporisation (ablation) include lasers, electrically heated wires or sample holders and electrical discharges (arcs, sparks).

Summary

After vaporization, solid samples may be swept into the centre of an argon plasma flame, where they are fragmented into ions of their constituent elements. The m/z values of ions give important information for identification of the elemental composition of the sample and measurement of ion abundances is used to provide accurate isotope ratios.

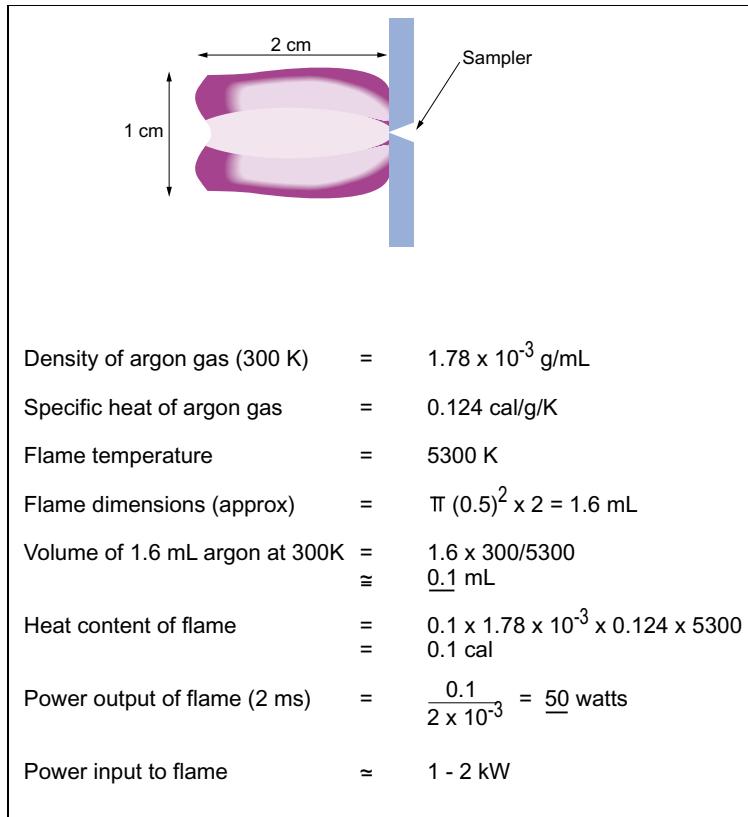


Figure 1. A plasma flame commonly has a diameter of about 1 cm and a length of about 2-3 cm. If this is regarded as being approximately cylindrical, the volume of the flame at about 5300 K is 1.6 mL and at 300 K is 0.1 mL. With a specific heat for argon of 0.124 cal/g/K and a density of 1.78×10^{-3} g/mL (at 300 K), the heat content of the flame is 0.1 cal. However, since gas flow through the hot flame occurs in a period of about 2 ms, the power output of the flame is about 50 watts. This should be compared with a power input from the high frequency electromagnetic field of about 1 kW. The seeming inconsistency between the high temperature and the low heat content arises because of the low number density of "hot" particles (the concentration of electrons and other particles in the hot flame is approximately 10^{-8} M).

SAMPLE INLETS FOR PLASMA TORCHES PART C: SOLID INLETS

Introduction To examine a sample by ICP-MS or ICP-AES, it must be transported into the flame of a plasma torch. Once in the flame, sample molecules are literally ripped apart to form ions of their constituent elements. These fragmentation and the ionisation processes is described in the Back-to-Basics guides, *Plasma Torches and Coronas*, *Plasmas and Arcs*. To introduce samples into the centre of the (plasma) flame, they must be transported there as gases or finely dispersed droplets of a solution or as fine particulate matter (aerosol). The various methods of sample introduction are described in the present guides in three parts (A,B,C) to cover gases, solutions (liquids) and solids. Some of these sample inlets can be multipurpose and can be used with, for example, gases and liquids or liquids and solids but others have been designed specifically for only one kind of analysis. However, the principles governing the operation of inlet systems fall into a small number of categories, which are described in these guides. This Part C deals specifically with substances, which are normally solids at ambient temperatures.

Problems of sample introduction

The two major difficulties facing the analyst/mass spectrometrist concern firstly how to get the whole of the sample into the plasma flame efficiently and secondly how to do so without actually putting out the flame or causing instability in its performance. Although plasma flames operate at temperatures of 6000-8000 K, the mass of "gas" in the flame is very small and its thermal capacity is correspondingly small (Figure 1). This means that, if a large quantity of sample is introduced into the flame over a short period of time, the flame temperature will fall and the basic ionisation processes leading to the formation and operation of the plasma itself are also interfered with. Therefore, introduction of samples into a plasma flame needs to be controlled and there is a need for special sample introduction techniques to deal with different kinds of samples. The major problem with introducing material other than argon into the plasma flame is that such additives may interfere with the process of electron formation, a basic factor in keeping the flame selfsustaining. If electrons are removed from the plasma by secondary processes faster than they can be replaced by the primary generation process then the plasma process ceases, viz., the flame goes out. Fluctuations in flame temperature and performance lead to significant accompanying variations in sample ion yield, often over short periods of time, and these fluctuations affect accurate measurement of isotope ratios. Thus, sample preparation and manipulation are

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important and, for any one type of inlet system, judicious choice of inlet conditions and sample preparation by the operator of the instrument can avoid the worst aspects of the problems just described.

Introduction of solids

It may be convenient to dissolve a solid so as to present it for analysis as a solution, which may be nebulized and "sprayed" as an aerosol (mixed droplets and vapour) into the plasma flame. This aspect of analysis is partly covered also in Part B, which describes the introduction of solutions. There are vaporisation techniques for solutions of solids other than nebulization but, since these require prior evaporation of solvent, they are covered here in Part C. There are many solid samples that need to be analysed directly and Part C describes commonly used methods for this purpose.

Basically, there is only one method for dealing with solids as such and that is to vaporize them in some way. Because solids vary from highly volatile, like iodine, to highly involatile, such as a ceramic material, it is not surprising that different methods have been devised for vaporising solid samples. Although desirable, it is often the case that a solid cannot be simply put into a vaporization chamber. For example, if a solid has been dissolved first in acid, it is necessary to remove excess of acid and/or solvent from the resulting liquid sample by selective heating so that the more volatile components are vaporized first and any solid residue is vaporized later.

The various heating methods produce a vapour that is a mixture of gas, very small droplets and small particles of solid matter (particulates). Before droplets or particulates can coalesce, the whole vapour is swept into the plasma flame for analysis. Clearly, the closer the heating source is to the sample flame, the less are losses due to deposition on surrounding walls in the instrument or on lead-ins to the flame. However, this does not prevent carriage of vapours over quite long distances in some cases (up to 20 m for example is possible for some inlets). The more important methods used for introduction of solids are based on lasers, arcs (and sparks) and on conventional electrical heating. In some instances, the sample may be heated directly by the plasma flame.

(a) Laser devices (Laser ablation, LA)

Laser desorption to produce ions for mass spectrometric analysis is discussed in the Back-to-Basics guide, *Laser Desorption*. As heating devices, lasers are convenient where a lot of energy is needed in a very small space. A typical laser power is 10^{10} watts/cm². Even when applied to a solid from a typical laser beam of a few tens of

Typical data for a laser running as a pulsed beam (Q-mode) could be:

$$\begin{aligned} \text{Power output} &= 10^9 \text{ watts.cm}^{-2} & = 10^9 \text{ J.s}^{-1} \text{ cm}^{-2} \\ \text{Pulse length} &= 15 \text{ ns} & = 15 \times 10^{-9} \text{ s} \\ \text{Laser beam radius} &= 0.5 \text{ m} & = 0.5 \times 10^{-3} \text{ cm} \\ \text{Area of sample exposed} &= \pi r^2 = 3.14 \times (0.5 \times 10^{-3})^2 & = 0.8 \times 10^{-6} \text{ cm}^2 \end{aligned}$$

Typical data for an iron sample could be:

$$\begin{aligned} \text{Density of iron} &= 8 \text{ g.cm}^{-3} \\ \text{Specific heat of iron} &= 0.1 \text{ cal.g}^{-1} \\ 1 \text{ J} &= 4.2 \text{ cal} \end{aligned}$$

Let ΔT = the rise in temperature (K), when an iron sample is heated by 1 laser pulse and a pit of 4×10^{-4} cm is produced.

Then,

$$\begin{aligned} \text{the volume of iron ablated} &= 0.8 \times 4 \times 10^{-10} \text{ cm}^3 & = 3.2 \times 10^{-10} \text{ cm}^3 \\ \text{the mass of iron ablated} &= 2.6 \times 10^{-9} \text{ g} \\ \text{heat required for ablation} &= 2.6 \times 10^{-10} \times \Delta T \text{ cal} \end{aligned}$$

But,

$$\begin{aligned} \text{heat input by laser in one pulse} &= 10^9 \times 15 \times 10^{-9} \times 4.2 \text{ cal.cm}^{-2} & = 63 \text{ cal.cm}^{-2} \\ \text{heat input over the ablated area} &= 63 \times 0.8 \times 10^{-6} \text{ cal} & = 5 \times 10^{-5} \text{ cal} \end{aligned}$$

If,

$$\begin{aligned} \text{heat input from laser} &= \text{heat used for ablation}, \\ 5 \times 10^{-5} &= 2.6 \times 10^{-10} \times \Delta T, \end{aligned}$$

And,

$$\underline{\Delta T} = 2 \times 10^5 \text{ K}$$

At 2% efficiency, estimated $\Delta T = 4000 \text{ K}$

Figure 2. The data shown in Figure 2 are typical of lasers and the sorts of samples examined. The actual figures used here are not crucial but serve to show how the stated energy in a laser may be interpreted as resultant heating in a solid sample. The resulting calculated temperature reached by the sample is certainly too large because of several factors, such as conductivity in the sample, much less than 100% efficiency in converting absorbed photon energy into kinetic energy of ablation and much less than 100% efficiency in the actual numbers of photons absorbed by the sample from the beam. If the overall efficiency is 1-2%, the ablation temperature becomes about 4000 K.

micrometres in diameter, this power can lead to very strong localised heating, which may be sufficient to vaporise the solid (ablation). Some of the factors controlling heating with lasers and laser ablation are covered in Figure 2. Suffice it to say at this stage that the surfaces of most solids subjected to such laser heating will be heated rapidly to very high temperatures and will vaporize as a mix of gas, molten droplets and small particulate matter. For ICP-MS, it is then only necessary to sweep the ablated aerosol into the plasma flame using a flow of argon gas; this is the basis of an ablation cell. It is usual to include a TV monitor and small camera to view the sample and to help direct the laser beam to where it is needed on the surface of the sample.

With a typical ablated particle size of about 1 µm diameter, the efficiency of transport of the ablated material is normally about 50%, most of the lost material being deposited on contact with cold surfaces or by gravitational deposition. From a practical viewpoint, this deposition in the ablation cell, the transfer lines and the plasma torch itself may require relatively frequent cleaning of the system.

There are different types of laser, which may be categorised according to the wavelength of the emitted radiation and by whether or not the lasers are used in a pulsed or continuous mode. Major characteristics of some commonly used lasers are given in the Back-to-Basics guide, *Laser Choices*, and this should be consulted for further details. For laser ablation, the choice of short-pulsed (Q-mode) or continuous (free-running) mode may be important for achieving a desired result but, in a practical sense, ultimate sensitivity of detection is not strikingly different, pulsed lasers generally giving some 10 times lower sensitivity. Pulsed lasers tend to give less total ablated material but this contains a greater proportion of gas to particulate matter. A proper comparison of the effects of pulsed and free-running lasers should take into account the total energy absorbed by the sample in unit time.

The degree of focussing of the laser beam is important. A tightly focussed laser beam delivers its energy to a very small area of sample so that the density of energy deposition is very high. This leads to the formation of a "pit" in the sample solid where material has been ablated. Successive pulses lead to the pit getting deeper and deeper. This mode of operation is used to produce a "depth profile", viz., a profile of the composition of a sample throughout its thickness. When the beam is defocussed, the area of sample irradiated is greater than in the focussed mode and the density of energy deposition is much lower. The resulting pit is very much shallower and covers a larger area. Therefore, the two kinds of beam use, focussed or defocussed, have different analytical consequences. In the focussed mode, the

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thickness of a sample is examined as the laser works its way down a deeper and deeper pit. The defocussed mode is mostly used to survey variations in sample composition across its surface (surface profile). The two methods of operation (depth and surface profiling) are complementary and each is useful in its own right.

A further important factor controlling the use of lasers for ablation purposes concerns the wavelength of the laser light and the sort of material irradiated. For ablation to occur, the sample should have one or more absorption bands overlapping the laser wavelength. The absorbed photons are converted rapidly into vibrational and rotational energy in the sample and, in turn, some of this internal energy is converted into kinetic energy, leading to material being ejected from the surface (ablation). If the sample does not absorb light at the laser wavelength, most of the laser beam will be reflected (or will pass right through a transparent sample) without causing any or much heating. If there is a substantial overlap of laser wavelength and absorption wavelength for the sample, much of the laser beam will be absorbed, with a concomitant rapid increase in temperature (about 10^{-7} to 10^{-8} seconds), with production of ablated material. At suitable infrared wavelengths, most substances have absorption bands and so the efficiency of absorption of energy can be quite high. However, at these longer wavelengths the energy of each photon is much less than at ultraviolet wavelengths. Therefore, with any one type of laser, the efficiency and amount of ablation can vary considerably from sample to sample but the variation tends to be less at infrared wavelengths.

In considering the use of a laser for ablative sampling in ICP-MS, major criteria that must be considered include, (i) laser power, (ii) pulsed or continuous laser operation, (iii) pulse repetition rate, (iv) focussed or defocussed laser modes, (v) depth or surface profiling, (vi) the absorption characteristics of the sample range. To obtain the greatest ablation yields, all of the previous factors should be optimised. Of these, the most difficult is the focussing and, for this reason, ablation cells normally have some sort of microscope to observe the surface of the sample continuously and to select the areas to be examined. With modern technology, it is more convenient and safer to use a CCTV camera to view the surface and a TV monitor to display the effects of the laser beam on the sample.

(b) Electrical discharge ablation

Under the right conditions, an electrical potential placed on two electrodes (anode and cathode) separated by a short distance (usually 1 to 5 mm) in a gas at normal pressures will lead to an electrical discharge as the insulating properties of the gas break down and a current flows between the electrodes. The discharge may be intermittent when it is called "sparking" or may be continuous when it is called "arcing". These processes are discussed in greater detail in

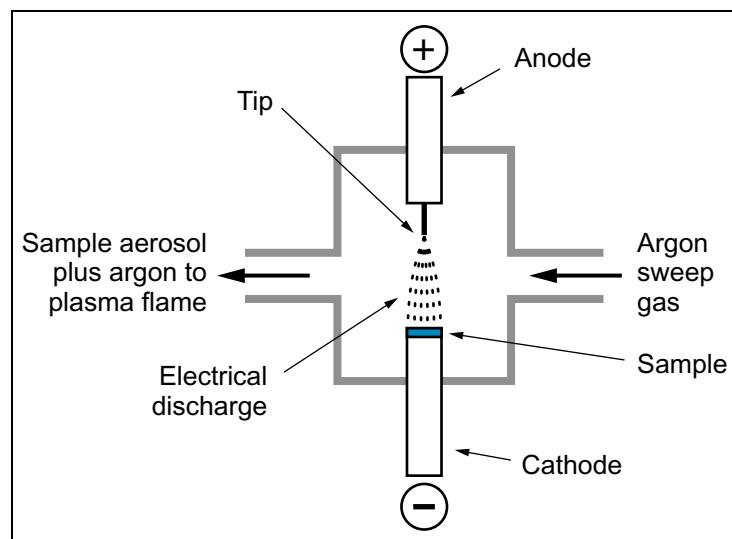


Figure 3. A schematic diagram showing the general construction of an arc or spark source. Actual constructional details depend partly on whether or not samples need to be analysed automatically. The sample material may be placed on the cathode or may even compose the whole of the cathode. If graphite is used, the sample needs to be pressed into the shape of a cathode after admixture with the carbon.

the Back-to-Basics guide, *Coronas, Plasmas and Arcs*. Generally, sparking occurs when the insulating properties of the gas between the electrodes is beginning to break down but the discharge cannot maintain itself for long periods. Conditions leading to this behaviour are low current flow associated with high voltages. Arcing occurs when the discharge becomes selfsustaining (more electrons are formed than are discharged at the anode) and electrically runs with a high current flow at low voltages. Sparks last for only a few micro or milliseconds but arcs may last for several minutes or more. In arcs or sparks, electrons flow to the anode and positive ions bombard the cathode so as to maintain overall electrical neutrality.

Because a spark or an arc have narrow diameters but contain a large number of ions and electrons, the heating effect caused by the current flow of a spark or an arc leads to the electrodes becoming very hot over a small area. The anode is usually shaped to a sharp "tip" to promote the discharge (compare the effect of a lightning conductor) and is cooled to prevent its temperature becoming too high; nothing is then ablated at this point. The heating effect of ion bombardment at the cathode leads to ablation of electrode material as particulate matter, molten droplets and vapour (an aerosol). Therefore, if a sample is included in the cathode material, it too becomes vaporised. The point of contact of the discharge at the cathode tends to wander over its surface and therefore any small heterogeneities in the sample are smoothed out during an analysis. As with the laser source discussed in (a) above, the ablated material is swept into a plasma torch for analysis of the elements present and their isotope ratios (ICP-MS). The argon gas, which is used to sweep the aerosol into the torch, prevents air getting to the sample being heated and therefore prevents oxidation or burning of the sample.

One problem with the spark or arc sources lies in sample preparation for non-conducting materials. If there is sufficient sample and it is conducting then it may be machined into a cathode or simply placed on the cathode surface (in electrical contact). Thus, this method of ablation is very useful for examination of metallurgical samples, which are normally conducting. For non-conducting samples, these must be thoroughly mixed with a conducting substance such as powdered copper or graphite and then pressed into a disc before being placed on the cathode. The added conducting material must be of ultra high purity so as not to introduce impurities into the analysis that are not in the sample itself. A typical generalised spark ablation source is shown in Figure 3.

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In operation, a spark source is normally first flushed with argon to remove loose particulate matter from any previous analysis and then the argon flow is reduced and the cathode is pre-heated or conditioned with a short "burn-time" (about 20 seconds) before the argon flow is reduced once more and the source is run for sufficient time to build a signal from the sample. The spark is then stopped and the process is repeated as many times as necessary to obtain a consistent series of analyses. The arc source operates continuously and sample signal can be taken over long periods of time.

Calibration of an arc or spark source is linear over three orders of magnitude and detection limits are good, often being in the region of a few microgrammes per gramme for elements such as vanadium, aluminium, silicon and phosphorus. Further, the nature of the matrix material composing the bulk of the sample appears to have little effect on the accuracy of measurement.

(c) Electrical heating
(Electrothermal
Vaporisation, ETV)

Electrothermal heating is basically the use of an electric current to heat up any suitable material, which may be in the form of a wire or filament (direct heating), a cup or in the shape of a small "oven". Whatever the kind of sample support, the sample to be examined is heated on the wire, in the cup or in the oven to a high enough temperature to effect its evaporation (usually between about 100 and 2000 °C). Volatile samples are dealt with easily by using a quartz holder, which is then heated externally. More generally, the sample is placed directly onto a filament prior to heating or put into a "boat", "cup" or "crucible", which is then heated. Sometimes a sample solution is placed in the boat or it is sprayed onto a thin graphite rod or into a graphite tube. After absorption of the sample solution, solvent is evaporated before residual solid sample is heated to vaporisation; the resulting aerosol is swept into the plasma flame for analysis. Typically, about 5-10 mg of sample are needed. Clearly, if the sample is already a solution (analyte dissolved in a solvent), the ETV device may be used as a liquid inlet (Part B) to examine the solution if the more volatile solvent is first evaporated at lower temperatures and then the residual analyte is vaporised at higher temperatures. To achieve variable heating, the electrical supply for ETV analysis is normally continuously variable.

Limits of detection for an ETV source are in the picogramme to nanogramme range and are often better than for direct solution introduction via a nebulizer. As with direct insertion methods (DSI, see below), graphite supports for solid samples often lead to carbide formation with difficultly volatile elements. In these cases, it is often the practise to add a "thermal reagent" to convert the sample

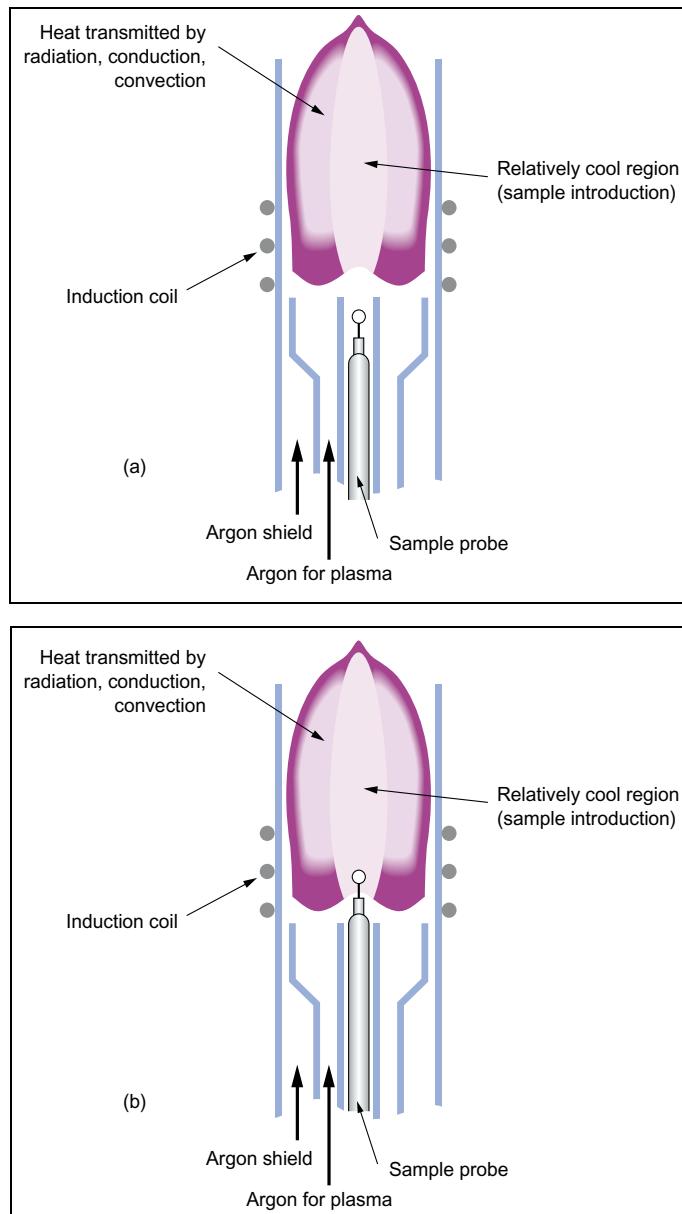


Figure 4. The diagram indicates the method of direct insertion of a sample into the plasma flame. Initially, the sample, held on a wire or in a small graphite or metal "boat", is placed just below the flame until conditions have stabilised. The probe is then moved up so that the sample holder enters the flame, where ablation occurs and the sample is simultaneously fragmented and ionised. The plasma flame is shown vertical instead of the usual horizontal so that the sample cannot run out or drop out of any cup that might be used for sample insertion. If there is no problem of containing or holding the sample then the flame can be in its more normal horizontal alignment.

elements into a more volatile form. For example, chlorine or fluorine containing compounds, such as halothanes may be added to the argon gas (about 2-5% by volume) or sodium chloride may be added directly to the sample preparation so that the solids react on heating. Interferences from oxide formation is very much reduced because there is no solvent passed to the plasma flame and air (oxygen) is kept out of the system.

(d) Direct sample insertion (DSI)

In principle, this is the simplest method for sample introduction into a plasma torch since the sample is placed into the base of the flame, which then heats, evaporates and ionises the sample, all in one small region. Inherent sensitivity is high because the sample components are already in the flame. A diagrammatic representation of a DSI assembly is shown in Figure 4.

In practise, direct insertion of samples requires a somewhat more elaborate arrangement than might be supposed. The sample must be placed on an "electrode" before insertion into the plasma flame; this sample support material is not an electrode in the normal meaning of the term since no electrical current flows through it. Heating of the electrode is done by the plasma flame. The electrode or probe should have small thermal mass so that it heats up rapidly and it must be stable at the high temperatures reached in the plasma flame. For these reasons the sorts of materials used for thermal ionisation sources in isotope analysis (see Back-to-Basics guide, *Thermal Ionisation*) may be used. Graphite is frequently the material of choice for the probe material both because of its good thermal properties and because it can be machined easily. However, graphite (carbon) forms carbides with many elements at high temperatures and these can lead to serious interferences in some instances. For example, the analysis of chromium at m/z 52 is made difficult by the formation of ArC^+ , which is also at the same m/z value. Other materials have been used, such as tantalum, tungsten or molybdenum. The probe may be in the form of a cup, in which to place the sample or it may be simply a thin wire loop, on which a sample solution has been evaporated so as to leave behind a solid residue. Probe temperatures of 2000-3000 °C have been measured.

These direct insertion devices are often incorporated within an autosampling device, which not only loads sample consecutively but also places the sample carefully into the flame. Usually, the sample on its electrode is first placed just below the load coil of the plasma torch, where it remains for a short time to allow conditions in the plasma to restabilize. The sample is then moved up into the base of the flame. This last movement may be made quickly so that sample

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evaporation occurs rapidly or it may be made slowly so as to allow differential evaporation of components of a sample over a longer period of time. The positioning of the sample in the flame, its rate of introduction and the length of time in the flame are all important criteria for obtaining good reproducible results.

Generally, sample sensitivities of several nanogrammes per gramme may be attained but precision may not be as good as with other introduction techniques. Volatile elements such as cadmium or zinc on a probe of small thermal mass are evaporated over a period of about one second, giving a sharp transient signal but slower evaporating elements give wider signals and sensitivity may not be so high. This variation in evaporation rate can even be used to achieve excellent sensitivity, as in the determination of volatile lead in non-volatile nickel.

Conclusion

Solid samples may be analysed through use of a plasma torch by first ablating the solid to form an aerosol, which is swept into the plasma flame. The major ablation devices are lasers, arcs and sparks, electrothermal heating and direct insertion into the flame.

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Back to Basics Section H: Miscellaneous

CHAPTER H1

EPA PROTOCOLS AND MS ANALYSIS

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Quick Guide

- The Environmental Protection Agency (EPA) authorizes control over hazardous and potentially hazardous substances and validates appropriate methods of analysis, many of which require GC-MS.
- The so called 'matrix' is the subject of any analysis, the predominant material of which the sample is composed. It is frequently either water or soil/sediment.
- The analysis programmes of the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) are managed through the EPA's Contract Laboratory Programme (CLP).
- In order for a laboratory to register under the CLP, certain test analytical procedures must be performed to the EPA's satisfaction.
- The official 'Series Methods' describe the exact procedures which must be followed when handling, preparing and analysing samples and reporting the results.
- Target compounds are specified for each Series Method.
- Some compounds which need to be analysed are volatile can be extracted from the matrix by a purge-and-trap device.
- Base, neutral and acid compounds, which may be less volatile, are extracted from the matrix with organic solvents.
- Only mass spectrometer-based analyses are discussed in this guide.
- Calibration and tuning of the mass spectrometer are achieved using either bromofluorobenzene (BFB) or decafluorotriphenylphosphine (DFTPP).
- Initial calibration for a Series Method is achieved by the analysis of a set of standards made up to specified concentrations.
- Continuing calibration for a Series Method is performed using calibration check compounds.

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- ‘Surrogate’ compounds are added to the matrix before sample preparation to evaluate recovery levels.
- To check GC retention times, internal standards are added to a sample after its preparation for analysis.
- The National Institute of Health - EPA mass spectral library is used to identify analysed components of a sample by comparing their mass spectra with those of authentic specimens held in the library.
- To produce a quantitative result, chromatographic peak areas of identified target compounds are compared with peak areas of the internal standards, which are of known concentration.

Summary The Environmental Protection Agency lays down strict guidelines for the analysis of a range of environmentally hazardous substances. Many of the analyses utilize GC-MS.

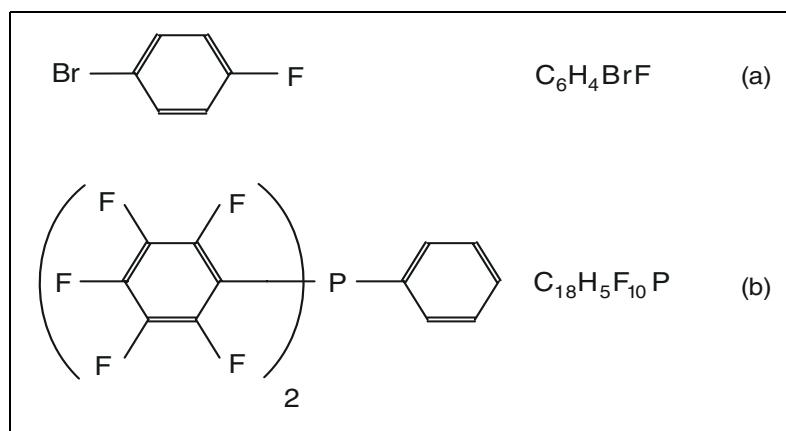


Figure I Chemical structures for BFB and DFTPP and their relative molecular masses (RMM; molecular weight):
(a) 4-bromofluorobenzene (BFB); RMM = 174, and
(b) Decafluorotriphenylphosphine (DFTPP); RMM = 442.

ENVIRONMENTAL PROTECTION AGENCY PROTOCOLS

Introduction

The Environmental Protection Agency of the United States (US EPA) exerts strict control over analysis procedures for monitoring, quantifying and recording a wide range of organic compounds which could cause environmental hazards if present at levels higher than those recommended.

The stringent control exerted by the EPA covers a good many application areas, with gas chromatography (GC) featuring prominently. The availability of computer-supported quadrupole mass spectrometry, its high intrinsic sensitivity, and its compatibility with gas chromatographs led to its adoption in the late 1970's as the primary tool for monitoring trace organics.

Environmental Laws

Complex environmental samples originate from diverse matrices (the predominant material of which the sample to be analysed is composed). These matrices, usually either water or soil/sediment, can contain as many as 50-100 organic components at widely varying concentrations. The EPA approach to the analysis of these samples involves the analysis of specific (or target) compounds and the use of authentic standards for quality control. The current number of standards in the EPA repository is about 1500, and their analysis is covered by various approved methods.

The EPA Contract Laboratory Programme (CLP) has responsibility for managing the analysis programmes required under the US Comprehensive Environmental Response, Compensation and Liability Act (CERCLA).

The approved analytical methods are designed to analyse water, soil and sediment from potentially hazardous waste sites to determine the presence or absence of certain environmentally harmful organic compounds. The methods described here all require the use of GC-MS.

The Contract Laboratory Programme

Certain laboratories can, after a contract has been awarded, register under the Contract Laboratory Programme (CLP) of the EPA. To earn a contract, one or more specifically prepared samples must be analysed under very similar conditions to those used in standard protocols. Only if the data are deemed satisfactory will a contract be awarded. Further evaluation samples must be analysed at three-monthly intervals afterwards to ensure that performance is being maintained.

Table I Ion abundance criteria for (a) bromofluorobenzene (BFB), and (b) decafluorotriphenylphosphine (DFTPP).

m/z	Ion abundance criteria for BFB	% Relative Abundance
50	15.0-40.0% of the base peak	24.1
75	30.0-60.0% of the base peak	50.5
95	Base peak, 100% relative abundance	100.0
96	5.0-9.0% of the base peak	7.4
173	Less than 2.0% of mass 174	0.3 (7.1) ¹
174	Greater than 50.0% of the base peak	50.6
175	5.0-9.0% of mass 174	3.6 (7.1) ¹
176	Greater than 95.0% but < 101.0% of mass 174	50.1 (99.1) ¹
177	5.0-9.0% of mass 176	3.5 (7.0) ²

¹Value in parenthesis is % of mass 174²Value in parenthesis is % of mass 176

m/z	Ion abundance criteria for BFB	% Relative Abundance
51	30.0-60.0% of mass 198	44.3
68	Less than 2.0% of mass 69	0.6 (1.3) ¹
69	Mass 69 relative abundance	43.3
70	Less than 2.0% of mass 69	0.6(1.5) ¹
127	40.0-60.0%of mass 198	57.0
197	Less than 1.0% of mass 198	0.0
198	Base peak, 100% relative abundance	100
199	5.0-9.0% of mass 198	7.0
275	10.0-30.0% of mass 198	24.7
365	Greater than 1.00% of mass 198	2.5
441	Present, but less than mass 443	10.3
442	Greater than 40% of mass 198	69.5
443	17.0-23.0% of mass 442	13.7 (19.8) ²

¹Value in parenthesis is % of mass 69²Value in parenthesis is % of mass 442

Protocols The Series Methods are used and these describe the exact procedures to be followed with respect to sample receipt and handling, analytical methods, data reporting and document control, and these must be followed closely, to ensure accuracy, reproducibility and reliability within and amongst the contract laboratories.

- a) 500 Series Methods The Safe Drinking Water Act (1974) controls the monitoring of finished drinking water, raw source water, or drinking water in any treatment stage. Method 524 monitors 60 purgeable (volatile) organic compounds in drinking water by the use of GC-MS. A purge-and-trap device is used to isolate the organic compounds by stripping them from water using a stream of an inert gas. The purged components are then trapped onto a porous polymer from which they can be thermally desorbed directly onto a GC column. Method 525 covers the analysis of 86 basic, neutral and acidic compounds in drinking water.
- b) 600 Series Methods The Clean Water Act (1972) requires discharge limits to be set on industrial and municipal waste water, and these analyses are outlined in the National Pollution Discharge Elimination System for the 600 Series Methods. Method 624 covers the analysis of purgeable organic compounds; Method 625 covers the analysis of 81 bases, neutrals and acids; Method 613 describes the analysis of dioxins and furans.
- c) 8000 Series Methods To satisfy the Resource Conservation and Recovery Act (1977) and its amendment for hazardous and solid waste (1984), the 8000 Series Methods have been designed to analyse solid waste, soils and ground water. In particular, methods 8240/8260 require the use of a purge-and-trap device in conjunction with packed or capillary GC-MS respectively for the analysis of purgeable organic compounds. Methods 8250/8270 concern analyses for the less volatile bases, neutrals and acids by GC-MS, after extraction from the matrix by an organic solvent.

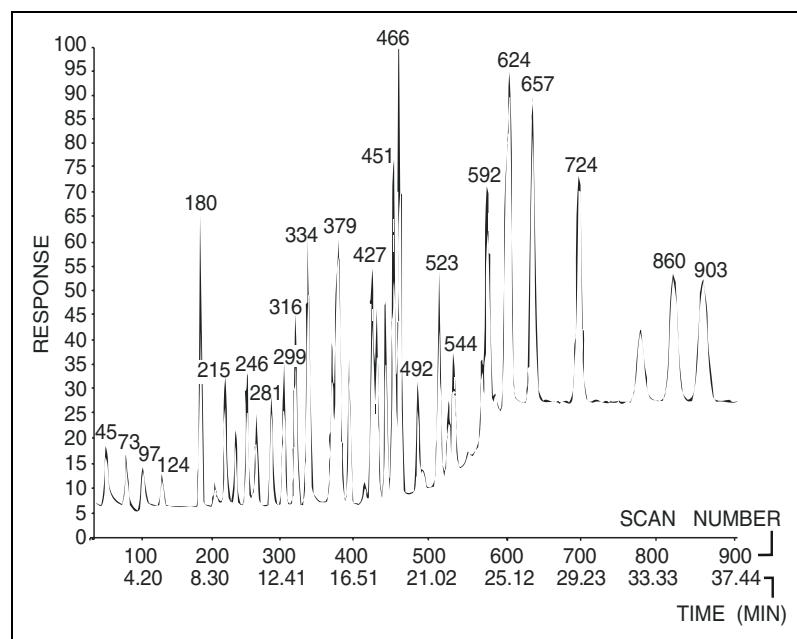


Figure 2(a) GC mass chromatogram showing the separation of a mixture of standards during initial calibration. The compounds corresponding to the peaks are identified in Figure 2(b).

Sample Analysis by GC-MS

Calibration Before sample preparation, the laboratory must demonstrate that the mass spectrometer is operating satisfactorily. First, the instrument must be tuned by calibration using one of two compounds. 4-Bromofluorobenzene (BFB) is used to establish tuning performance prior to the analysis of purgeable organic compounds and decafluorotriphenylphosphine (DFTPP) is used prior to the analysis of bases, neutrals and acids (Figure 1).

The positive ion electron ionization spectra of BFB and DFTPP must exhibit molecular and specified fragment ions, the relative abundances of which must fall within a pre-defined range. Ion abundance criteria for BFB and DFTPP are shown in Table 1. This calibration procedure, which must be demonstrated at the start of each working period (or 12 hour shift) ensures that all samples are analysed with respect to a known reference point of the mass spectrometer.

The next step is to show that the response for the analysis of any target compound is linear. This is known as the initial calibration and is achieved by the analysis of standards for a series of specified concentrations to produce a five point calibration curve (Figure 2 a, b). On subsequent days, a continuing calibration must be performed on calibration check compounds to evaluate the calibration precision of the GC-MS system.

Sample Analysis Before sample preparation, surrogate compounds must be added to the matrix. These are used to evaluate the efficiency of recovery of sample for any analytical methods. Surrogate standards are often brominated, fluorinated or isotopically labelled compounds which are not expected to be present in environmental media. If the surrogates are detected by GC-MS within the specified range, this is taken to be an indication that sample preparation and analysis have been performed satisfactorily. If the surrogates are out of specification, re-analysis or even re-preparation of the sample is required.

Interim report			Initial calibration			06-FEB-87 17:14:24		
No.	Rank	Spectrum	Scan	Peak	Figs	Scan	Quan	Compound name
		Match	Diff	Area		Found	m/z	
1	99	60	99	0	2404000	bb	260	Bromochloromethane
2	86	44	93	0	7130000	bb	332	1,2-Dichloroethane-d4
3	89	44	99	0	7178000	bb	45	Chloromethane
4	94	51	99	0	3706000	bb	73	Bromomethane
5	93	50	99	0	6428000	bb	97	Vinyl Chloride
6	83	34	99	0	3418000	bb	124	Chloroethane
7	100	79	99	0	11963000	bb	180	Methylene Chloride
8	72	33	99	0	2778500	vv	200	Acetone
	52	12	99	8	495000	?v	208	
	47	4	98	-9	131000	??	191	
9	100	78	99	0	17782000	bb	215	Carbon Disulphide
10	100	69	99	0	4451000	bb	246	1,1-Dichloroethane
11	98	58	99	0	11006500	bb	281	1,1-Dichloroethane
12	100	68	99	0	5588500	bv	299	1,2-Dichloroethane (to
13	100	76	99	0	13830000	bb	316	Chloroform
14	91	49	96	0	10453000	bb	335	1,2-Dichloroethane
15	90	45	99	0	16378000	bb	523	1,4-Difluorobenzene
16	78	25	99	0	2427652	bv	334	2-Butanone
17	100	69	99	0	10077000	bv	368	1,1,1-Trichloroethane
18	92	48	99	0	9371000	vb	378	Carbon Tetrachloride
19	80	46	82	0	21057960	vb	380	Vinyl Acetate
20	100	64	99	0	10350460	bv	393	Bromodichloromethane
21	100	72	98	0	9990000	bb	427	1,2-Dichloropropane
22	100	64	98	33	11815000	bb	466	cis-1,3-Dichloropropen
23	100	66	98	0	6840000	bb	446	Trichloroethane
24	79	30	96	0	6787000	bb	465	Dibromochloromethane
25	73	41	96	0	6394530	bv	467	1,1,2-Trichloroethane
26	100	74	97	0	28387000	bb	458	Benzene
27	83	35	97	-33	11249710	vb	433	trans-1,3-dichloroprop
28	91	47	99	0	4062000	bb	536	Bromoform
29	67	2	47	0	4509000	?b	630	Chlorobenzene-d5
	28	3	46	2	29000	??	632	
30	76	23	98	0	8146502	bb	812	4-Bromofluorobenzene
31	88	41	99	0	18205590	bv	619	Toluene-d8
32	98	57	99	40	10291170	vv	584	4-Methyl-2-Pentanone
33	88	42	98	-40	7731600	vb	544	2-Hexanone
34	94	53	98	0	5249000	bb	591	Tetrachloroethane
35	88	41	99	0	9299502	vv	594	1,1,2,2-Tetrachloroethane
36	96	55	98	0	16024560	bv	624	Toluene
37	88	41	99	0	17310850	bv	659	Chlorobenzene
38	89	45	98	0	10301000	bb	724	Ethylbenzene
39	83	34	98	0	19074000	bb	860	Styrene
40	82	32	99	0	11223000	bb	902	m-Xylene
41	57	32	99	4	10456000	bb	906	o-/p-Xylene

Figure 2(b) An interim report on the analysis of the mixture of standards illustrated in Figure 2(a).

Internal standards at a known concentration are added to the sample after its preparation but prior to analysis to check for GC retention time accuracy and response stability. If the internal standard responses are in error by more than a factor of two the analysis must be stopped and the initial calibration repeated. Only if all the criteria have been met can sample analysis begin.

A considerable amount of time is necessary to reach the point at which sample analyses can commence and it is essential that the stability and reliability of the mass spectrometer is high to ensure maximum sample throughput during the limited time available between calibration checks.

Data Processing

After analysis, target compounds (those compounds which the analysis is aiming to detect) are recorded by the data processing facilities of the mass spectrometer (Figure 3). The correct location of target peaks in a gas chromatogram is verified by the use of target compound databases, stored by the data system of the mass spectrometer. These databases hold information pertaining to the target compounds: name, retention time, search window with respect to the retention time, and so on.

Once chromatographic peaks have been assigned to target compounds, the various mass spectra from the sample are compared with library spectra of the target compounds; a match between an observed and a library spectrum serves to verify the identification of a component (Figure 4). The closeness of the match is given a score and the higher the score the better the identification. The National Institutes of Health (NIH)-EPA mass spectral library contains over 44,000 entries.

Chromatographic peak areas are calculated automatically by the data system by reference to the response obtained from certain, specified compound-dependent ions. From the peak areas of the target compounds, quantification is achieved by comparison with the internal standards, which are present in known concentration. The laboratory responsible for the analysis must report the target compounds and all tentatively identified (non-target) compounds. Standard EPA forms must be completed and submitted. A laboratory is said to be in compliance when it has satisfied all aspects of its CLP contract.

Interim report Sample Analysis							06-FEB-87 18:35:36			
No.	Rank	Spectrum Match	Spectrum Fit	Scan Diff	Peak Area	Figs	Scan Found	Scan Pred	Quan m/z	Compound name
1	100	79	99	1	2005000	bb	210	209	128	Bromochloromethane
2	83	41	97	-1	5365000	bb	267	268	65	1,2-Dichloroethane-d4
3	100	248	99	-4	2262000	bb	32	36	50	Chloromethane
4		No Trace Found					59	94		Bromomethane
5		No Trace Found					78	62		Vinyl Chloride
6		No Trace Found					100	64		Chloroethane
7	92	84	99	-5	17767000	bb	140	145	84	Methylene Chloride
8		No Trace Found					161	43		Acetone
9		No Trace Found					174	76		Carbon Disulphide
10		No Trace Found					199	96		1,1-Dichloroethane
11	100	76	99	-1	36246000	bb	226	227	63	1,1-Dichloroethane
12		No Trace Found					242	96		1,2-Dichloroethene (t)
13		No Trace Found					255	83		Chloroform
14		No Trace Found					270	62		1,2-Dichloroethane
15	87	59	99	3	14924000	bb	426	423	114	1,4-Difluorobenzene
16		No Trace Found					272	72		2-Butanone
17		No Trace Found					300	97		1,1,1-Trichloroethane
18		No Trace Found					308	117		Carbon Tetrachloride
19	86	63	81	-1	23897000	bv	309	310	43	Vinyl Acetate
20		No Trace Found					320	83		Bromodichloromethane
21	100	85	99	-3	33719000	bb	345	348	63	1,2-Dichloropropane
22		No Trace Found					353	75		cis-1,3-Dichloropropen
23		No Trace Found					363	130		Trichloroethene
24		No Trace Found					379	129		Dibromochloromethane
25		No Trace Found					380	97		1,1,2-Trichloroethane
26		No Trace Found					373	78		Benzene
27		No Trace Found					380	75		trans-1,3-Dichloroprop
28		No Trace Found					445	173		Bromoform
29	36	0	57	0	20730000	bb	482	482	117	Chlorobenzene-d5
30	100	65	99	0	14070500	bb	646	646	95	4-Bromofluorobenzene
31	97	62	99	1	27585000	bb	504	503	98	Toluene-d8
32		No Trace Found					447	43		4-Methyl-2-Pentanone
33		No Trace Found					416	43		2-Hexanone
34		No Trace Found					452	164		Tetrachloroethene
35		No Trace Found					455	83		1,1,2,2-Tetrachloroethane
36		No Trace Found					477	92		Toluene
37		No Trace Found					504	112		Chlorobenzene
38		No Trace Found					554	106		Ethylbenzene
39		No Trace Found					658	104		Styrene
40		No Trace Found					690	106		m-Xylene
41		No Trace Found					693	106		o-/p-Xylene

Figure 3 Identification of some target compounds in an environmental sample. The report lists the closeness of match and the estimated quantities for the listed compounds.

Conclusion Through sets of ‘Series Methods’, the US Environmental Protection Agency describes procedures for the detection and estimation of environmentally hazardous substances. There are strict requirements for accuracy, reproducibility and for calibration of mass spectrometers.

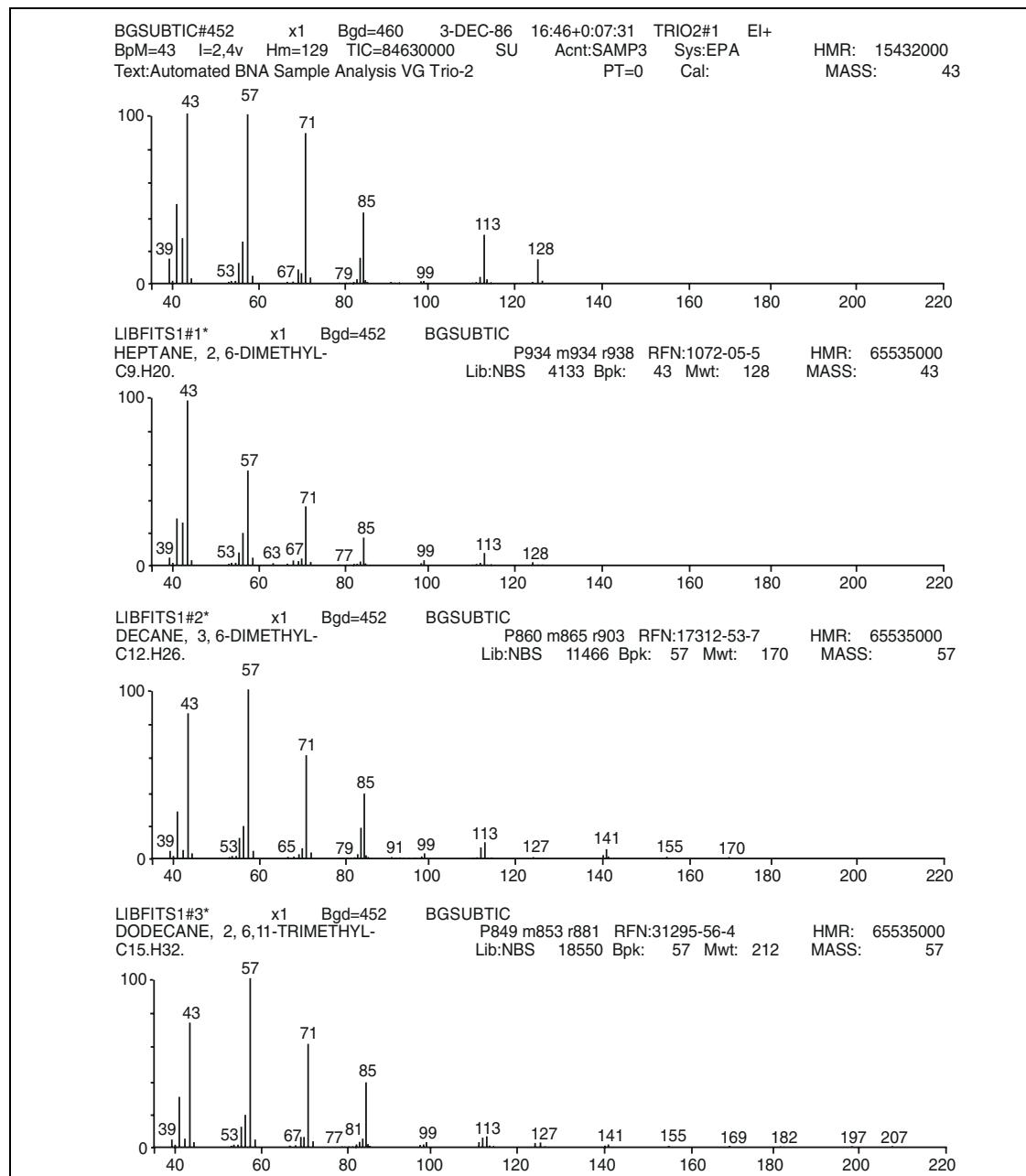


Figure 4 Comparison of the mass spectrum from a target compound (top), with the three best fits from the library of standard spectra (lower three traces). The closeness of fit of the mass spectra and the chromatographic retention time lead to a positive identification of 2, 6-dimethylheptane.

Back to Basics Section H: Miscellaneous

CHAPTER H2

COMPUTERS AND TRANSPUTERS IN MS, PART A

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Quick Guide

- Binary numbers are composed of strings of zeros and ones. A typical binary number is 1110 which is equivalent to the decimal number 14.
- Binary numbers (just two digits) are useful in computer construction because the zero can be represented by an electronic switch in the off position and the one by a switch in the on position. Movement (change) from zero to one or vice versa is then simply the change in a switch from off to on or vice versa.
- All information into or out of a computer is therefore not continuous (analogue) but flows as bits switched on and off (digital), viz., like a series of electronic pulses.
- The special electronic switches are called bits and are arranged in sets, sometimes referred to as registers or memory locations, depending on usage. The sets of bits are 8 in the simplest computers, 16 in more advanced ones and 32 or 64 in the latest.
- Whether there are 8, 16, 32 or 64 bits in each set, the set is called a byte.
- The capacity of a computer to carry out various tasks is partly governed by the number of bytes it has. Thus, a one megabyte memory means there are one million locations with 8 or 16 or 32 or 64 bits in each.
- A computer must be able to organize communication with peripheral devices (keyboard, mouse, printer, mass spectrometer) and to do this it has a central processing unit (CPU), rather like a choreographer directs complicated dance routines.
- Some of this organisation is completely automatic and is called the operating system. Generally, it cannot be altered by the user and it uses a low level machine language (machine code) which helps it to respond quickly to commands.
- The instructions for the operating system reside within a memory unit which can be read but not changed in any way - the so-called read only memory (ROM).

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- The user can add his own commands by way of a programme (software) which the central processor will carry out. These programmes reside in memory, usually on a disk (either hard or floppy) rather than the processor's own memory banks.
- These memory units must be capable of change as necessary for running software programmes. They are known as random access memory (RAM).
- Most computers have far more RAM than ROM and the more they have the more tasks can be carried out.
- The software programmes are usually written in a more user friendly high level language such as Fortran or Pascal or C because machine programming is quite tedious and demanding of effort.
- Although much easier to assemble, a high level software programme requires more time for the computer to execute because all the instructions must be translated into machine code before the computer can understand them. Even a simple statement like 'start' in a high level language requires several machine code moves to execute.
- The working of the CPU is controlled by a crystal clock having a frequency of 16 to 25 MHz generally, depending on the type of computer. All electronic 'moves' are controlled by the clock and operate in sequence to its 'ticking'.

Summary Digital computers operate with a binary system whereby all operations basically consist of a series of on/off electronic switching controlled by a crystal clock. The smallest on/off unit is the bit and these are assembled into larger units known as bytes. The various functions of a computer are controlled through a processor, the CPU, which deals with incoming and outgoing signals and the execution of instructions (software) dealing with the signals.

$$\begin{array}{r} 1 \quad 7 \\ 2 \quad 4 \\ \hline 1 \\ 1 \quad \rightarrow \end{array} \qquad \begin{array}{r} 1 \quad 7 \\ 2 \quad 4 \\ \hline 4 \quad 1 \\ \hline \end{array}$$

Figure 1 Addition of two decimal numbers. Note the carry-over of one ten from the rightmost column into the left one.

$$\begin{array}{r} 1 \quad 7 \\ 2 \quad 4 \\ \hline 4 \\ 1 \quad \rightarrow \end{array} \qquad \begin{array}{r} 1 \quad 7 \\ 2 \quad 4 \\ \hline 4 \quad 4 \\ \hline \end{array}$$

Figure 2 Addition in the heptimal system. Now, one seven is carried from the rightmost column into the left hand side one.

COMPUTERS/TRANSPUTERS PART A

Introduction An understanding of computer and transputer operation requires some basic knowledge of the working of a microprocessor. This simplified account of the use of computers and transputers begins with some discussion of the fundamentals of computing. It must be emphasised that the following account is very elementary and books on computing should be consulted for a deeper understanding.

The account begins with binary arithmetic, moves on to on-off (flip-flop) electronic switches, then to serial and parallel processing and finally to computers/transputers.

Multibase Arithmetic Much of modern life revolves around the use of the decimal system of numbers although, for many purposes, it is far from ideal. This system (dec = ten) takes ten as the basic unit of operation and we say that we are working to *base ten*. However, the base ten is not the only one possible; the previous British and many other countries' coinage was founded on a base of twelve. The meaning of a base for purposes of calculation is illustrated in Figure 1. If two numbers (17 and 24) are to be added together then the calculation proceeds as follows: $7 + 4 = 11$ but 11 is $10 + 1$ and so a 1 is written down and one ten is added to the next column. In the next column there is one ten from the 17, two tens from the 24 and one ten 'carried' over and the total number of tens is $1 + 2 + 1 = 4$. Therefore, the answer is 41 - but *only* in the decimal system! Suppose the calculation is done on a base of seven - a heptimal system. Then, repeating the above calculation, $7 + 4 = 11$ as before but now, 11 is *one* 7 with 4 left over. Therefore, one 7 is carried to the left (Figure 2) and the 4 left over is written down. In the next column, there are one seven not one ten (as with decimal 17) and two sevens (from the 24) making a total of three sevens; the total number of sevens is then $3 + 1$ (carried from the previous column) = 4 and this is written down. Thus, the total obtained from the addition of 17 and 24 in the two systems is 41 in one and 44 in the other! Which is correct? In fact, we have cheated a little because 17 (decimal) written in the heptimal system should be two sevens and *a* three (23) and the 24 should be *three* sevens and *a* three (33). It is 23 and 33 which must be added: $3 + 3 = 6$ (less than 7 and so 6 is written down, with no seven to carry into the next column) and the next column is $2 + 3 = 5$, giving an answer of 56 in a fully heptimal system.

(a)	1000	100	10	1
	or, 10^3	10^2	10^1	10^0
	thus, $3(1000)$	$2(100)$	$6(10)$	$5(1) = 3265$ (decimal)
(b)	8	4	2	1
	or, 2^3	2^2	2^1	2^0
	thus, $1(8)$	$0(4)$	$0(2)$	$1(1) = 1001$ (binary)

Note: The sequence of columns in binary runs 1, 2, 4, 8, 16, 32, 64, 128, 256, 512, 1024, 2048, 4096 and so on, each column being two times greater than the previous one. Compare this with the decimal system where each column is 10 times greater than the previous one.

To convert 1001 into a decimal number we have to take 1×8 plus 0×4 plus 0×2 plus 1×1 which is 9 (decimal). Conversion of 3265 into binary is a little more difficult: the largest binary number near 3265 is 2048 (or 2^{11}) and there is only one of them with 1217 left over; the next largest binary number is 1024 (or 2^{10}), leaving 193; the next binary number is 128 (or 2^7) and then 64 (2^6) and 1 (2^0). Thus, the binary equivalent of 3265 is 110011000001.

$$\begin{array}{r}
 3265 \\
 -2048 \quad (1 \times 2^{11}) \qquad \qquad \qquad 1 \\
 1217 \\
 -1024 \quad (1 \times 2^{10}) \qquad \qquad \qquad 1 \ 00 \ (2^9, 2^8) \\
 193 \\
 -128 \quad (1 \times 2^7) \qquad \qquad \qquad 1 \\
 65 \\
 -64 \quad (1 \times 2^6) \qquad \qquad \qquad 1 \ 00000 \ (2^5 \text{ to } 2^1) \\
 1 \quad (1 \times 2^0) \qquad \qquad \qquad 1
 \end{array}$$

Figure 3 (a) In the decimal system, a number like 3265 means $3 \times 1000 + 2 \times 100 + 6 \times 10 + 5 \times 1$.
 (b) In the binary system, a number such as 1001 means $1 \times 8 + 0 \times 4 + 0 \times 2 + 1 \times 1$. Note how the columns are arranged in ascending powers of the base, 10 for decimal and 2 for binary.

The above results appear most confusing but only if the bases are mixed. Working with one base all the time (e.g. decimal) there is no confusion.

Binary Arithmetic

Two systems have been illustrated above, one in use in every day life and the other just a mathematical curiosity employed to emphasise that it is not necessary to work always to the base of ten. To use the decimal base, ten numbers are needed: 1, 2, 3, 4, 5, 6, 7, 8, 9 and 0. All other numbers are made up from these. In the binary system ($b_{ini} = 2$) there are only two numbers, viz., 0 and 1 and all numbers can be made up from just these two. We need to know that $2^0 = 1$, $2^1 = 2$, $2^2 = 4$, $2^3 = 8$ and so on. Just as decimal addition was arranged in ones and tens and hundreds ($10^0 = 1$, $10^1 = 10$, $10^2 = 100$, etc.; Figure 3) so the binary system can be arranged in the same way. In binary code, the number 3 is a 1 ($1 \times 2^0 = 1$) plus a 2 ($1 \times 2^1 = 2$) and is written **11** (Figure 3). Similarly, the number 17, must now be written $2^0 (= 1)$ plus $2^4 (= 16)$ or, in binary code, **10001** ($1 \times 2^4 + 0 \times 2^3 + 0 \times 2^2 + 0 \times 2^1 + 1 \times 2^0$; Figure 4).

Simple arithmetical operations in binary code are easy in that only two numbers are used (0,1). An example of adding 17 and 24 in binary code is shown in Figure 5. The answer is 101001 and the other decimal numbers (2 to 9) are unnecessary. The penalty lies in the complexity! Imagine remembering that the decimal numbers 1, 2, 3, 4, 5, 6, 7, 8, 9 are 1, 10, 11, 100, 101, 110, 111, 1000, 1001 and trying to work out your change after purchasing something, even ignoring all the other numbers. A 999 call becomes 1111100111. However, it turns out that, for machines, a base of two is highly desirable because only two fundamental operations are needed, viz., ‘off’ or ‘on’ corresponding to ‘0’ and ‘1’. As far as a machine is concerned, the number 3 (decimal) which in binary is 11 means setting two ‘switches’, both ‘on’. If the machine can sense that a switch is off or on then it can carry out arithmetical operations in binary language.

2^0	2^1	2^2	2^3	2^4	2^5	2^6	2^7	2^8	2^9
1	2	4	8	16	32	64	128	256	512

What is the binary equivalent of decimal 5? The largest power of 2 which fits 5 is $2^2 = 4$. Therefore there is 1×2^2 in 5 with 1 left over; $2^1 = 2$ which is too big and so we write 0×2^1 ; finally we see that $1 \times 2^0 = 1$. The number 5 is made up from a 1×2^2 and a $1 \times 2^0 = 1$ but in binary we must not forget to put 0×2^1 ; in decimal we may write 304 meaning three hundred and four and if the 0 had been omitted then the number would have been thirty four (34). Thus, the binary equivalent of 5 is **101** and not **11**. Other numbers may be converted into binary in an exactly similar fashion. Decimal 39 is:

$32 + (0 \times 16) + (0 \times 8) + 4 + 2 + 1$ which is **100111** in binary.

Figure 4 To convert a decimal number into binary it is necessary to look at the powers of 2 as described in Figure 3. Decimal numbers corresponding to ascending powers of 2 are shown here, up to 2^9 . Each number is just twice the previous one.

Addition of 17 and 24:

$$17 \text{ (decimal)} = 10001 \text{ (binary)}$$

$$24 \text{ (decimal)} = 11000 \text{ (binary)}$$

$$\underline{\underline{41}} \text{ (decimal)} = \underline{\underline{101001}} \text{ (binary)}$$

Figure 5 Addition of two numbers in binary code. Note the carry over in the left most column caused by adding two ones.

Electronic Switching and Binary Code

There is an electronic circuit called a 'flip-flop'. It consists of two transistors connected in such a way that, if a voltage is applied, one side of the circuit becomes active and the other side not; if a second voltage is applied, the circuit flips so that the active side becomes inactive and vice versa. Thus, just as with a conventional switch for which one 'touch' puts it on and a second 'touch' turns it off, one touch of the flip-flop turns it 'on' and a second touch turns it 'off'. Addition of two binary numbers now becomes possible. Suppose we want to add $2 + 1 (=3; \text{decimal})$. First, the numbers must be converted into binary code (10 and 01) and these become switch settings in the machine but we need four switches so that 10 becomes on, off and 01 becomes off, on (Figure 6). If the first pair of switches is examined, one is off and the other on and the result of 'touching' each must be a resulting 'on' (off -- on and on -- off, giving a 'total' of 'on'). For the other pair exactly the opposite sequence is present but the net result is 'on'. As far as the machine is concerned, the result is 'on, on' which in our binary code is 11 and this in decimal code is 3, the correct answer. Therefore, to get the machine to add in binary, it is necessary to have a switch for each power of two that we want. The number 2^6 is 64 (decimal) and, to represent any number up to 63, we must have seven switches (seven flip-flop circuits), viz., $2^5, 2^4, 2^3, 2^2, 2^1, 2^0$ and zero. In computer jargon these switches are called *bits*. Normally, a 'rack' of switches comes in multiples of eight or sixteen or thirty two or sixty four. A *byte* refers to the whole 'rack'. Thus, 8 bits make a byte or 16 bits make a byte and so on (Figure 7). A megabyte of computer memory in 8 bit binary code will have 8,000,000 switches!

A consequence of this mode of handling is that the electronic signals into or out of or inside the computer flow as series of tiny pulses with gaps between, corresponding to the on/off switching of the bits. For this reason they are known as *digital* devices because of their relation to the two binary numbers used (two digits). For it to be usable, a continuous (analogue) electrical signal must be *digitized* before it can be used, viz., its continuous nature must be split up into a series of discrete pulses.

Switches (1,2)	On Off equivalent to	1	0
Switches (3,4)	Off On equivalent to	0	1
Add	On On equivalent to	1	1

Figure 6 An electronic switching circuit may be ‘on’ or ‘off’ and these positions are used to represent the two basic binary numbers 1 and 0 respectively.
Decimal 2 is 1 0 in binary (switch settings on, off) and decimal 1 is 0 1 in binary (switch settings off, on).

Registers

The various parts of a computer have different functions to deal with peripherals (the viewing screen, the mouse, the keyboard and inputs and outputs for transmission of data) and all of these must be controlled by a *central processor*. In this guide these functions are not considered. The processor also supports an arithmetic unit which enables addition, subtraction, multiplication and division to be carried out. To do this, the processor has a number of registers which are racks of bits. A typical 8-bit register is shown in Figure 8. Let this be called the x-register. Underneath is a similar y-register and then an a-register. The x- and y-registers are now each filled with a number that we want to add. Suppose these are 17 and 24 (decimal). Seven of the bits are used to represent 2^0 to 2^6 , viz., to represent the numbers 1, 2, 4, 8, 16, 32, 64; the last bit is for ‘carry over’. Number 17 (decimal) is made up of 1×16 plus 1×1 and in our 7-bit binary code becomes 0010001 (binary). The ‘switches’ or bits in the x-register are set to off off on off off off on. Similarly, 24 (decimal) becomes 1×16 plus 1×4 or 0011000 (binary) and this is entered into the y-register. To add them is a simple matter: two zeros (two offs) are still zero; a zero and a one (off and on) are one; two ones become zero and carry one to the left (**on** plus **on** is zero but turns **on** the next bit along). The sum of this addition is shown in Figure 8 where we see the result 0101001 (note that there has been a carry over from bit 5 to bit 6). In decimal numbers, 0101001 means $0 \times 64 + 1 \times 32 + 0 \times 16 + 1 \times 8 + 0 \times 4 + 0 \times 2 + 1 \times 1 = 41$ (which is correct for $17 + 24$!).

Operations such as the above are carried out very rapidly by the computer through voltage switching, each switch lasting only a few nanoseconds. Therefore, although it is clumsier to represent numbers in binary for the human mind and instead we use ten symbols (0, 1, 9) to help us with complicated arithmetic, the speed with which we can do this is nothing like the speed of the computer. Computer addition seems instantaneous whereas we all know that human response to addition takes a finite time.

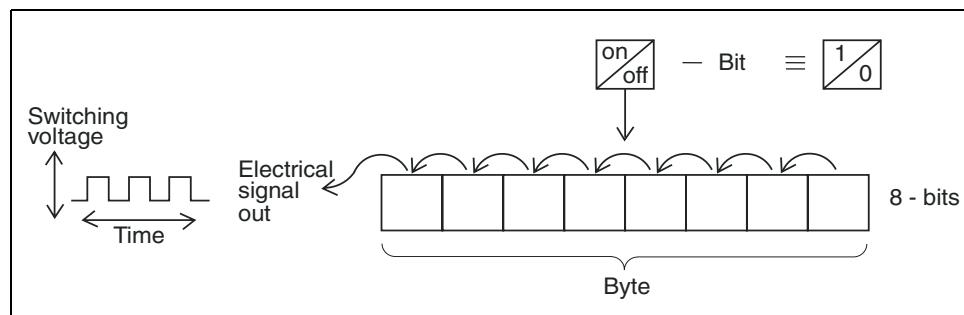


Figure 7 Bits are put together as bytes. In this example, it is an 8-bit byte. Faster, more powerful computers have more bits to the byte (16, 32, 64). In 'reading' a byte, the bits flow one after the other out of the byte as electronic pulses (a positive voltage for 'on' and zero for 'off').

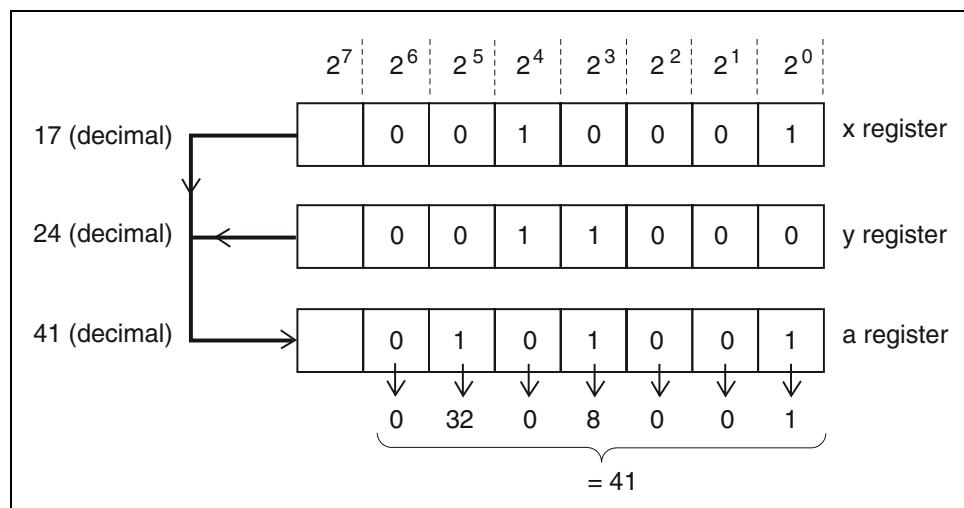


Figure 8 Two decimal numbers (17, 24) represented in binary bits in an x-register (0010001) and a y-register (0011000). 'Addition' into the a-register gives 0101001 which translates as 41 (decimal).

Most of the above discussion has concerned addition. Subtraction in binary is very similar but multiplication is awkward (try it!). For this reason it is quicker for a computer to multiply by carrying out a series of additions. Multiplying 3×5 becomes adding $5 + 5 + 5$ and so on. Because each addition is very fast then the time taken for even a large multiplication is very little and still appears instantaneous to us. Only with very large computations does this speed become obvious enough to merit special computers, more powerful than the ones being considered here for use in mass spectrometry. Finally, division is very like multiplication except that a series of subtractions is carried out instead of additions.

Other Registers

The above discussion has concentrated on arithmetical operations by computing in binary numbers represented as bits and bytes. However, other computer functions also use bytes of information. For example, in word processing, all the letters, symbols, punctuation marks and so on must be coded to reside in memory and the memory itself must be organized. Most letters and so on are represented by a standard coding (ASCII code) in which each is given a byte value. For example, the binary code for a capital letter 'M' is 1001101. The computer must be able to differentiate between this value meaning capital M and its arithmetic value (77 decimal) and to do this 'flags' are used. The flags are simply reserved bits which the computer uses as a guide. Similarly, there must be flags to tell the computer to stop or start and other flags so that the computer knows where to look in its memory for various items. Each memory location is a byte for which there is a flag or addressing pointer.

All operations of the computer processor or memory banks revolve around the use of bytes of information in which some of the bits carry the essentials and one or two other bits in each byte carry directional or instructional flags. A central processor of a computer is a piece of hardware which has been pre-programmed with its own memory (not accessible to the user - the so-called ROM or read only memory) and is used to organize the reception of information (input), initial processing of the information, sending on the initial information to a store (memory) or a software programme for further processing and finally sending out results or instructions (output).

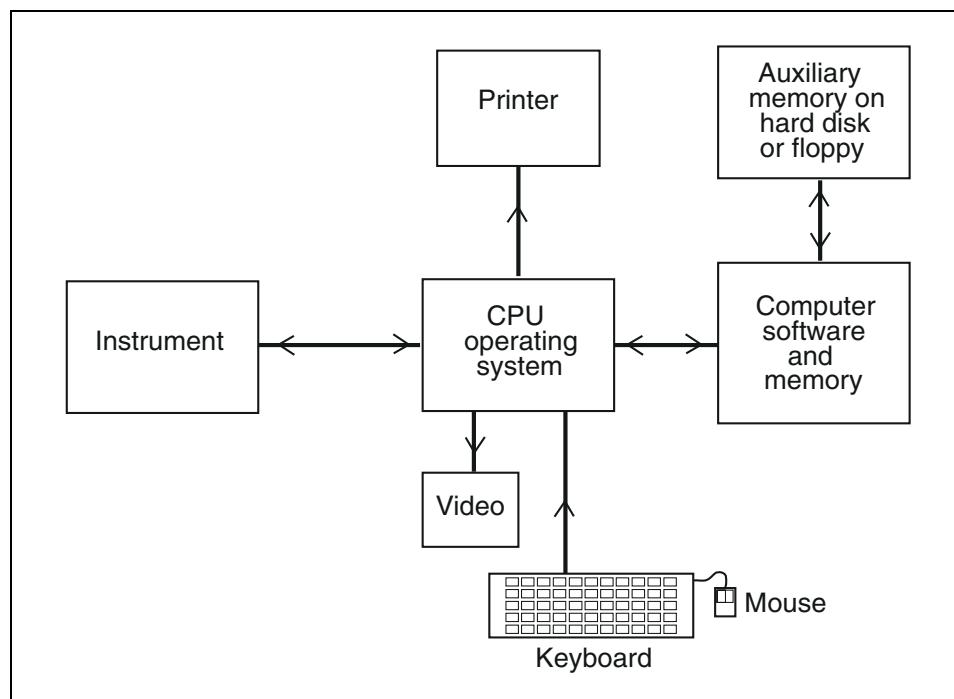


Figure 9 A typical layout controlled by the central microprocessor (CPU). Electrical inputs are received from the keyboard or mouse or instrument. Outputs go to the video screen, printer and the instrument. Memory and software are utilized by the CPU on command.

The input may be from a PC (Personal Computer) as in word processing but could equally well be from an instrument; the output could be to a video screen, a printer or to the same or another instrument (Figure 9). All these functions are organized by the central (micro)processor in so-called *real-time*, i.e., virtually immediately.

Bits and Bytes

A simple 8-bit device has been described above. These are very limited in what they can handle. For example, in terms of numbers, an 8-bit device (1 byte) alone can only deal with numbers up to 127 or 255, depending on how the eighth bit is used. This would not be much use for general work although it might be sufficient for simple instrument control. To get to larger numbers other bytes must be used in conjunction with each other. Use of several bytes together necessarily slows down computation. To get around this problem, larger sized bytes are used. The first major step was the introduction of first 16-bit devices and then the now common 32-bit bytes. There are even available 64-bit bytes. The latter can deal directly with numbers up to about a thousand million and is very much faster than having to string together four or five 8-bit bytes. There is a price to be paid. Constructing 8-bit byte devices (chips) is now *relatively* straightforward but even so, the yield of perfectly functioning chips is not good and many have to be thrown away. For the 64-bit chips the engineering complexities are enormous and the yield of perfect devices is quite low - hence the high cost. Except for very large 'number-crunching' computers, most general purpose ones such as the PC work with 16- or 32-bit chips.

Languages

A computer must be instructed in the operations it has to carry out. Although some instruction is built into the computer and appears as 'hardware' (the physical construction) most of the instruction set is written in a suitable language and then the computer is *programmed* to do its job. At the lowest level of language, the so-called machine code, instructions are written in a very elementary fashion and, literally, each step of the instruction sequence must be spelt out. For example, in every day life one might say something like 'draw a square'. This would be high level language. In machine code, the same instruction might be 'put a dot on the video screen at location x, y; move horizontally to the right for z squares; stop; move vertically down for z squares; stop; and so on'.

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Programming in machine code is slow, skilled work but has the advantage that any instruction to do something is carried out at the fastest possible speed. Thus, where speed is important, as in the central processor unit (CPU), all instructions are built permanently in machine code and usually cannot be altered by the person using the computer. The CPU has its own memory, instructions on what to do in the event of a command (e.g., pressing a key on the keyboard is a command), and directions for dealing with a variety of signals (as from an external instrument). For many other peripheral purposes instructions are written in high level languages. The instruction, 'draw a square' could be a statement to the computer in some high level instruction set. Actually, this statement would be translated into low level machine code automatically and, although on the face of it only simple, readily understood (by humans) instruction has been given, in fact, it has been quietly converted (translated) into machine code by the programming language itself so that the computer can 'understand' it. All high level languages are actually indirect means of providing programmers with a less tedious way of instructing a computer than through the direct use of machine code. Therefore, once having got a basic set of instructions built into a central processor to enable it to deal quickly with electronic impulses (signals) from keyboards, instruments, printers and so on, other instructions can be written as a programme (software) in a convenient high level language which can be loaded into the computer's main memory and which the computer will obey once started. Clearly, if one instruction in a high level language is made up of say 20 machine level instructions (and usually they are many more!) it will take 20 times longer to carry out that 'simple' instruction like 'draw a square'. For this reason it takes a computer much longer to process data when its instructions (the programme) are written in high level languages. Nevertheless, this disadvantage is more than made up for by the relative simplicity of writing instruction sets in a high level language. Typical high level languages are Fortran, Pascal, C and Occam.

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Computer Memory

As set out above, certain parts of computer memory are reserved exclusively for the central processor and other parts for driving any peripherals such as a keyboard. Although this memory can be accessed (read) it cannot be changed and hence its name of read only memory (ROM). Other memory is usually provided for the user by having a chunk of memory which can be changed by either the user directly or indirectly via a programme in the computer. These memory locations can be changed at will and hence the name, *random access memory* (RAM). The amount of RAM in a computer is usually quoted in megabytes (1 Mbyte = 1 million locations or memory registers) and the more there is available the more complicated the tasks that can be tackled. A typical PC might have 20 - 200 Mbytes of memory available and be able to support several software programmes. Simpler computers such as those aimed mainly at the games market have much more modest amounts of memory.

The Clock

All the instruction sets in the computer could not operate without some form of timing device being available because the length of time needed by the various instructions changes with the instruction. The problem could be exemplified by reference to control systems such as traffic lights. These are operated on a timer basis and, without them, traffic would soon jam at busy periods as each vehicle (instruction) tried to get to its destination (memory location) across the paths of others. Of course the clocks used with computers need to 'tick' at a very fast rate if the instructions are to be carried out quickly. For example, a clock ticking at the rate of 20,000,000 times per second (20 MHz), can deal with each basic instruction in about 0.00000005 seconds. These clocks are not the usual kind found with clockwork mechanisms but consist of special crystals which oscillate constantly at these high rates when an electrical voltage is applied to them rather like the modern 'quartz' watches. They are called crystal clocks. The 'ticks' are not mechanical ones but consist of electrical impulses which, during the 'on' time allow something to proceed and in the 'off' period stop anything from happening. Computer instructions 'go by the clock'.

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Conclusions By electronic engineering, a system of interconnected switching devices is able to respond in one of only two modes (on or off) and these modes can be controlled at the basic level of a bit. Bits are assembled into bytes, as with an 8-bit device, and through programming of the bytes a computer central processor can be made to follow sets of instructions (programmes) written in special languages, either at a direct level (machine code) that can be acted upon immediately by a computer or at a high level which is translated for the user into machine code.

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COMPUTERS AND TRANSPUTERS IN MS, PART B: A COMPARISON OF THE TWO

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Quick Guide

- Movement of information (operations) in a computer is controlled by a (micro)processor.
- In conventional processors, the various operations are carried out sequentially, viz., one after the other in a strictly controlled succession of movements (serially).
- The total time taken to carry out a sequence of operations (or instructions) is the average time for one operation multiplied by the number of operations. To carry out 100 instructions, each taking 100 nanoseconds, requires a total of 10,000 nanoseconds.
- An alternative to serial execution of a computer working is to split up the total work into smaller groups with each group carrying out its function simultaneously with the others (in a parallel fashion), viz., flows of instructions run in parallel (at the same time as each other).
- Now, if the average time needed to effect one instruction is 100 nanoseconds and there are 100 such operations but handled by 10 groups of processors working in parallel, then the time taken is only 1000 nanoseconds: $(100/10) \times 100$.
- Parallel working is inherently faster than serial processing but special processors are needed and these are called transputers.
- Parallel processing requires that each transputer be able to communicate with others (up to four immediate neighbours with present transputers) if the final result is not to be garbled.
- Each transputer is a microprocessor with its own memory banks and its own built in operating mode similar to a 'normal' microprocessor but additionally has input and output channels to enable it to communicate with other transputers. For example, in one simple mode, five transputers could be coupled together so that four of them were carrying out operations at the same time (in parallel) but controlled by the fifth.
- A special computer language (Occam) is needed to enable transputers to be programmed in this co-operative mode, yielding true parallel processing of information with all its advantages in speed.

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- Additionally, transputers have a reduced number of operating instructions available (RISC set) and this means that any sequence of operations is quicker to carry out because the processor does not have to search through a large instruction set in order to find out exactly what to do (compare reading this Quick Guide with having to search a textbook on computers).
- Parallel processing and the RISC set have given transputers a considerable speed advantage over conventional serial processors for handling information or flows of data.
- Because of the past extremely large investment in time and resources in developing serial processors and their software programmes they are unlikely to be dropped in favour of transputers in the near future. However, in situations in which large amounts of information must be handled in a very short time, transputers hold a very distinct advantage over conventional processors and are being used.
- A mass spectrometer with its need for acquiring data and processing it at high speed whilst simultaneously handling instrument control or printing or library searching (foreground/background working) is one such application where transputers are revolutionising the situation and it is no surprise to find them being used increasingly in mass spectrometer systems.

Summary

By carrying out a set of instructions in parallel rather than sequentially (serially), any total operation (a set of computer programmes) can be carried out much faster. The transputer is a special microprocessor designed to work in parallel with other transputers, using its own special programming language (Occam) and, to make the total operation faster still, using a reduced set of basic instructions.

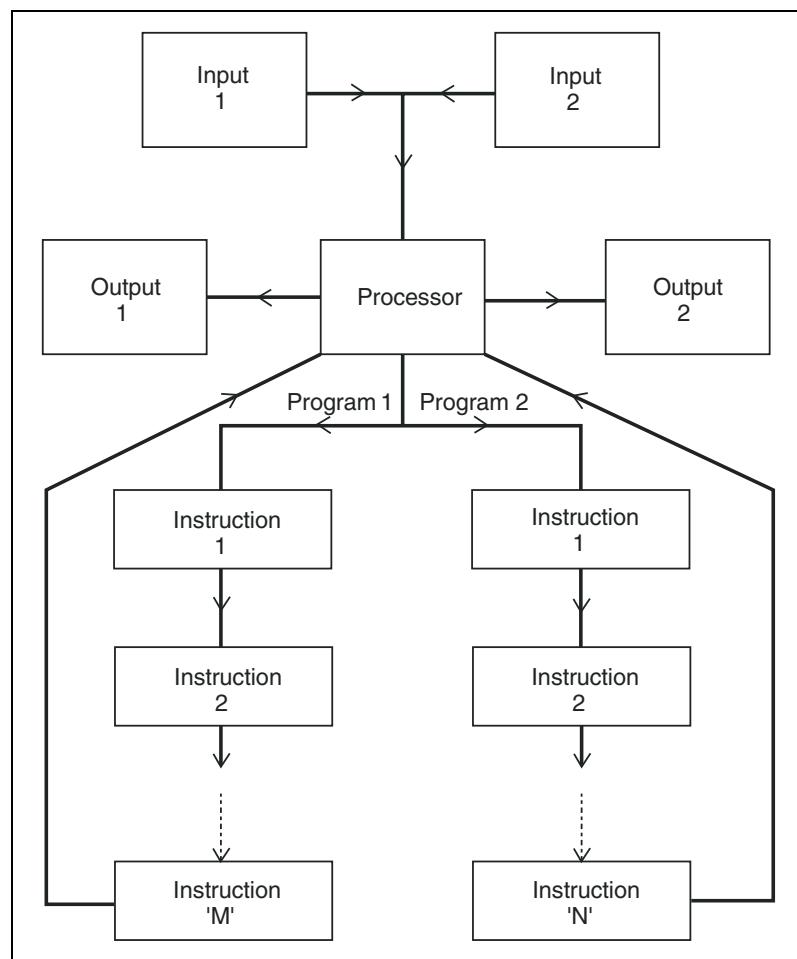


Figure 1 A very simple illustration in which information from two inputs is dealt with sequentially (serially) by a microprocessor. Input 1 is accepted and the left-hand series of instructions (programme 1) are carried out. Then, Input 2 is examined and the right-hand set of instructions is followed through. The processes are iterated. If each programme (1, 2) takes 1 msec, the total time for one iteration is 2 msec.

COMPUTERS/TRANSPUTERS, PART B: A COMPARISON OF THE TWO

Introduction

Many of the terms used in this Guide have been introduced in Part A and it is assumed that the reader will have read Part A or be familiar with its contents.

Serial and parallel processing are two different ways in which information is passed around a computer system. 'Normal', present day, computing has relied on serial methods whereby each snippet of information follows another in a logical fashion until the end result is arrived at (Figure 1). Most software programmes developed up until now rely on a serial approach in which the total set of instructions is laid out one after the other and no part of the programme can proceed until its turn arrives. A great deal of effort and time and money has gone into production of processors, construction of software programmes and development of hardware for serial computing and will not be lightly jettisoned. Nevertheless, when it comes to speed of processing information, the parallel mode has many advantages and this has given rise to a new type of computer called a transputer (Figure 2). The present guide discusses in a brief fashion the advantages to be gained from the use of transputers in acquiring and processing data from an instrument like a mass spectrometer which needs to deal with large scale input and output at high speed.

Basic Speed Differential between Parallel and Serial Modes

Movement of information in a computer could be likened to a railway system. Carriers of information (bits or bytes) move together (like a train and wagons) from one location to another along electronic tracks. It is important that no two bits of information get mixed up and therefore all the moves have to be carefully timed in step with a clock. This resembles the movement of trains on a railway; many trains use the same track but are not all in the same place at the same time. The railways run to a timetable in a similar way to that by which information is moved around the computer under the control of the central processor (CPU). Consider a process which requires 100 pieces of information to be moved around - this could be part of a calculation, a bit of word processing, direction of data inputs and so on.

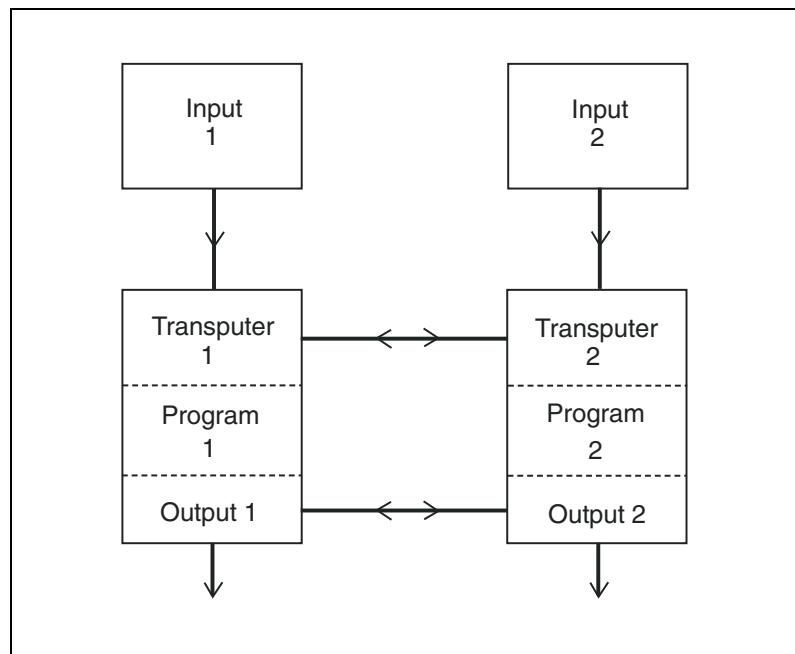


Figure 2 Compared with Figure 1, there are two transputers, each dealing with a set of instructions. If the processing time for each is 1 msec, the total time required is still about 1 msec. Communication links are needed between the transputers.

Suppose the average time taken to process each piece of the information is 100 nanoseconds then the total time taken for 100 pieces of information is 10,000 nanoseconds or 10 milliseconds. This is very fast compared with the railway and to a human is 'instantaneous'. However, 100 pieces of information is not much and might only be equivalent to putting say 10 characters onto a screen. When it comes to dealing with information entering the computer at a high rate, as when a mass spectrum is being recorded, this speed of moving information is not fast enough, as will be described in Part C of these guides. The major problem arises from the need to move the all the information sequentially on the same electronic 'track' (called a data bus). The speed with which the information moves is one factor, the other is the need to control its flow which all goes through the CPU along the bus which turns out to be a bottleneck (like the time taken on a railway is governed partly by the speed of a train and partly by the need to stick to a timetable so as not to have collisions between trains which are running on the same track). One way out of this 'bottleneck' problem is to provide more routes for the information to flow on (parallel processing), just as one way (very expensive) of speeding up a railway would be to have many more lines. In a computer these extra lines can be provided but a different way of timetabling the movement of information is needed. This is where the transputer comes in.

Suppose the above example of 100 pieces of information is taken but, instead of dealing with it serially, there is provided a network of 10 electronic tracks. Now the time taken to move this information is only 1 millisecond, viz., the system is ten times faster than the serial one. Simply providing 10 new tracks is not an answer in itself. Each track must be provided with its own microprocessor to deal with the information and the processors must be able to communicate with each other so that the information flows in an orderly fashion (it is no use having two different train tracks if each train arrives at the same platform at exactly the same time!). Thus, the transputer is a microprocessor which has its own memory bank and input and output lines enabling it to communicate with other transputers in a parallel fashion.

For example, one transputer could deal with data input into a mass spectrometer (recording a mass spectrum) whilst another could be controlling the actual scanning of the mass spectrometer; the two would work in conjunction in that, once scanning had been instituted by one transputer, the other would record information until told to stop by the first.

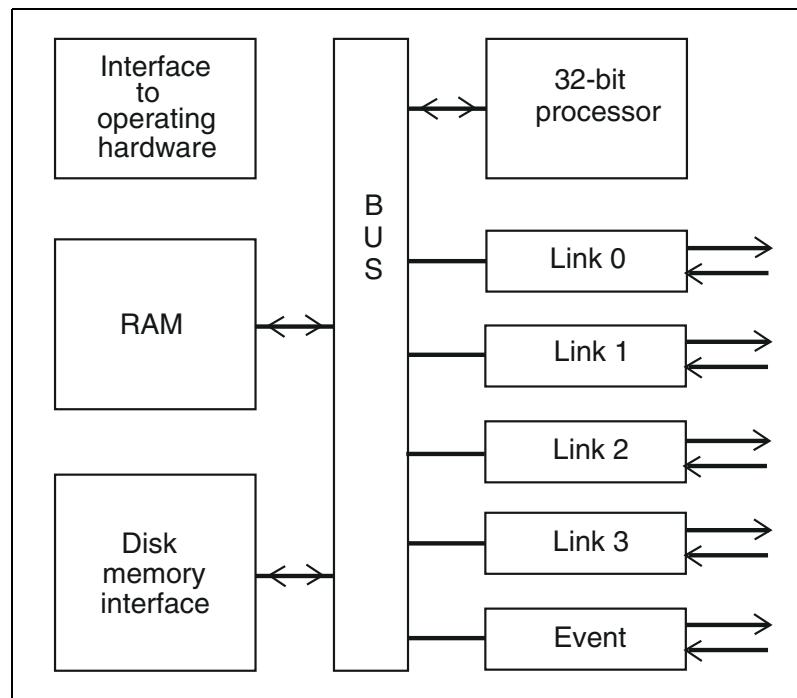


Figure 3 A typical transputer ‘architecture’. The transputer (sometimes referred to as a computer on a chip) has four input/output links (0, 1, 2, 3) to other transputers, a channel for inputting/requesting data (event link), some built-in random access memory, an interface to the main operating system (clock, boot, etc.) and an external memory interface. Internal communication is via a bus.

Parallel processing of information has led to a speeding up of computer operations without having to try to push the limits of existing technology by attempting faster and faster processors. A typical outline of transputer architecture compared with that of a standard computer is shown in Figure 3. Because the transputer has a 32-bit processor and considerable random access memory to which access is very fast, it has been called a 'computer on a chip'. Transputers are inherently faster than microprocessors which have to refer for RAM outside the chip on which they reside. Thus, the 100 nanosecond cycle time used by way of illustration above may be only 50 nanoseconds when carried out on the transputer 'chip' compared with a standard microprocessor having to refer 'off-chip' for RAM.

This big increase in speed has not been without cost. The everyday machine codes and high level languages (Fortran, Pascal, C, etc.) used to control operations in a standard computer are inappropriate for parallel processing which needs its own instruction set and has led to the simultaneous development of special languages along with that of the transputer.

- Occam** The most developed language for controlling transputer operations has been called *Occam* in honour of the philosopher William of Occam who, along with other minimalists, maintained that there is no point in trying to describe anything with more than the bare minimum of facts and that added supposition is superfluous and should be chopped out (hence the term *Occam's Razor*). The language, Occam, enables programmes to be written in parallel mode in which instructions can be passed from transputer to transputer. Thus, one transputer can acquire and process information but will then wait for a signal from another transputer before passing on the result to possibly a third transputer or to memory or to a peripheral. Each transputer has four input and four output lines so that it can communicate directly with up to four other transputers. Occam allows control of these inputs and outputs.
- This language allows groups of transputers to operate together, each carrying out a specific task and with access to its own memory bank, and then sending forward the processed information in harmony with the operations of the other transputers. This timetabling would get round the difficulty of the two trains arriving at the same platform at exactly the same time - one would be held back slightly until the other had cleared the platform.

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Because of the past effort that has been invested in other high level 'serial' languages and the large number of useful software programmes written in these languages, it is unlikely that transputers and Occam will simply take their place. Where there is a very clear advantage in being able to handle high rates of flow of information and process it at high speed then transputers will be used more and more, as in mass spectrometry. Where the application doesn't need the high speeds attainable with the transputer (word processing for example), it is unlikely they will be given high priority application. Because most computer programmers are not conversant with parallel programming and are likely to be reluctant to learn yet another language, there has been some attempt at adapting Fortran and Pascal to deal with parallel processing by adding some additional parallel-working instruction sets. This approach also eases the 'marriage' of two different approaches (serial and parallel) to processing where both are used in the same application.

There is another way in which the transputer has been speeded up relative to common computer operations and this is through a reduction in the number of basic instruction sets available to the programmer and this aspect is discussed next.

Reduced Instruction Set for Computing (RISC)

A high level language like Fortran makes programming a computer to carry out a set of operations much easier than if machine code is used directly. Thus, operations like add, subtract, multiply, divide, logarithm, sine, cosine, go to a defined place, return from a defined place, repeat a sequence and so on are all available as simple written statements in a high level language. What is not readily apparent is that the processor in the computer must translate these statements into machine code before the computer can carry them out. An instruction to a computer as simple as 'add' may require 10-20 machine code operations.

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Whereas there is not much difference in the execution time for carrying out an instruction directly in machine code or indirectly after translation there is a definite delay involved whilst the computer looks for the translation. If 'add' is one instruction amongst say a compilation of perhaps a couple of thousand others then each time the computer has to carry out this instruction it has to search through this large set to find the one it wants. If there is only a small set in the first place then the time taken is greatly reduced (at the same time the high level language becomes more unwieldy in a general sense in that the programmer has to formulate some of the operations such as *sine*). For many computer operations, the provision of a large instruction set is 'overkill' in that many of them will be used rarely if at all by the application to which the language is being applied. This circumstance applies to most data acquisition and processing applications for instruments like mass spectrometers and it makes sense in such applications to reduce the number of instructions for which the computer has to search. Therefore, processors have been designed with a reduced instruction set (RISC), making them faster in moving information around. The transputer was designed from the start with a RISC 'vocabulary', making it inherently faster than processors having a full instruction set, with or without parallel processing.

Conclusion

Microprocessors (transputers) have become available which, with the help of a special language (Occam), can handle flows of information in a parallel fashion instead of sequentially (serially) thereby making any operation much faster to carry out. Transputers can communicate with each other to control the flow of information.

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COMPUTERS & TRANSPUTERS IN MS, PART C: THEIR USE WITH MASS SPECTROMETERS

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Quick Guide

- Most electrical signals flowing from or to a mass spectrometer to or from an attached computer are of the analogue type, viz., the associated voltage varies continuously with time.
- Inside a computer, electrical signals consist of series of pulses in which voltage rises from zero to a maximum and back to zero in a very short space of time (a pulse). These are digital voltages.
- For a mass spectrometer and a computer to operate together, the analogue and digital voltages must be interconverted through the use of either an analogue-to-digital converter (ADC) or a digital-to-analogue converter (DAC).
- The mass spectrometer provides a mass spectrum which is actually an analogue voltage varying in amplitude with time as ions of different m/z values arrive at the ion collector in a very short period of a few seconds.
- After passing through an ADC, the resulting large number of 'digits' or bits of information must be reduced to a more manageable level before being passed on to the computer storage area.
- This reduction in information is achieved by a pre-processor which uses the digital voltages corresponding to an 'ion peak' to estimate the peak area (ion abundance) and centroid (mean arrival time of peak, equivalent to m/z value); these two pieces of information plus a 'flag' to identify the peak are stored.
- By working with a pre-processor, what would have been say a million bits of information needing to be stored becomes only some 300.
- Having acquired the mass spectral information, various software programmes can be employed to print out a complete or partial spectrum, a raw or normalized spectrum, a total ion current chromatogram, a mass chromatogram, accurate mass data and metastable or MS/MS spectra.
- Apart from acquiring data, a computer can be used to tune the mass spectrometer and to check that all systems are operating correctly. This working mode is carried out by sequentially examining various input voltages from different parts of the instrument, checking these against 'normal' readings and altering any which need to be adjusted.

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- Thus, a computer attached to a mass spectrometer must operate on two levels. When mass spectral information is arriving, this has to be acquired in 'real time'. As and when it has 'spare' time, the computer controls the operation of the instrument. These operations are carried out at such a high speed that the two level working is not obvious.
- However, the two levels may become obvious if the instrument operator tries, for example, to carry out a library search whilst the computer is trying to acquire input from another mass spectrum - the library search has to wait. Acquiring the data is foreground working. Running other functions such as library searching is background working.
- Powerful mass spectrometer/computer systems can achieve simultaneous foreground/background working, especially if transputers are used so as to take advantage of parallel working.

Summary A computer attached to a mass spectrometer is used both to acquire data and to control the operation of the spectrometer. Powerful transputer systems can be used to ensure that these two modes of working can be carried out almost simultaneously.

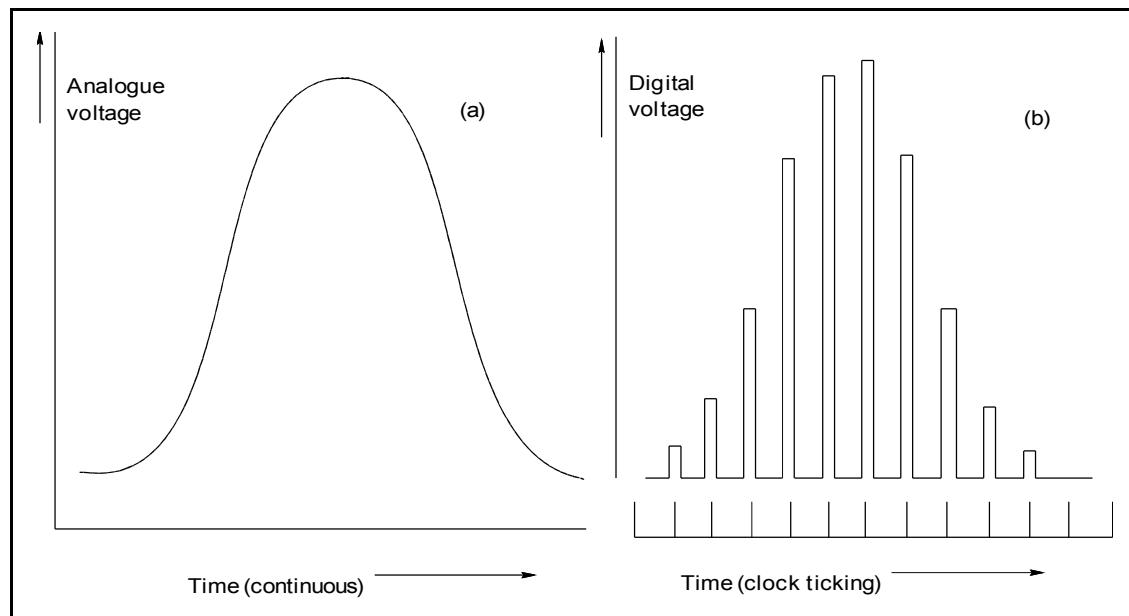


Figure I (a) An analogue voltage signal representing a mass spectral peak varies continuously with time, rising through a maximum and back to the baseline.
(b) In a digitized form, a clock 'ticks' and, on each tick, the analogue voltage is read out. Thus, the peak now appears outlined as a series of discrete digital readings rather than in continuous form.

COMPUTERS/TRANSPUTERS, PART C: THEIR USE WITH MASS SPECTROMETERS

Introduction Brief outlines of the workings of computers and transputers have been presented in Parts A, B of this Guide and they should be read before this Part, unless the reader is already familiar with the basics of computing. It will also be found that more detail on some of the functions is available in other Guides of this series. Where this is the case, cross-references are given.

From the point of view of computation, a mass spectrometer is a source of data which must be acquired, processed, stored and printed but it is also an instrument which must be tuned and controlled in its operations. In this Guide the two types of operation are covered separately. However, for both types, it is generally true that most electrical signals reaching the computer input are *analogue* ones which is to say they are continuous (Figure 1a) but the computer or transputer is a digital device, *viz.*, its electrical signals are of the form of pulses (Figure 1b). Therefore, before the analogue signal can be accepted by the computer it must be digitized and, for this, an *analogue-to-digital converter* (ADC) is used. Similarly, any signal coming from the computer is in digital form and must be turned into an analogue signal by a *digital-to-analogue converter* (DAC). Interconversion of analogue to digital and vice versa is exemplified, in Figure 2, by reference to a voltage varying with time. In the later discussion in this Guide it will be assumed that the electrical signals are in their correct form through use of ADCs or DACs as necessary.

Data Processing A mass spectrum consists of a series of peaks at different m/z values with the height of the peak being proportional to the number of ions. A partial mass spectrum is shown in Figure 3 and is seen to be an analogue signal which varies as the peaks rise from and fall to the baseline. Between the peaks are relatively long intervals when there is only the baseline. As described above, the signal is first digitized. If one imagines a clock ticking, then each time it ticks a measure of the peak height is read (actually as a voltage). Figure 4 gives a representation of this process. The clock 'ticks' very fast at something like 100,000 times per second and therefore a stream of voltage readings flows to the computer. These large amounts of data are 'indigestible' and would rapidly fill the available memory. Accordingly, there is a pre-processor which reduces the amount of information. First, an artificial baseline is set (Figure 5) else otherwise the natural noise on the true baseline would appear as even more small peaks.

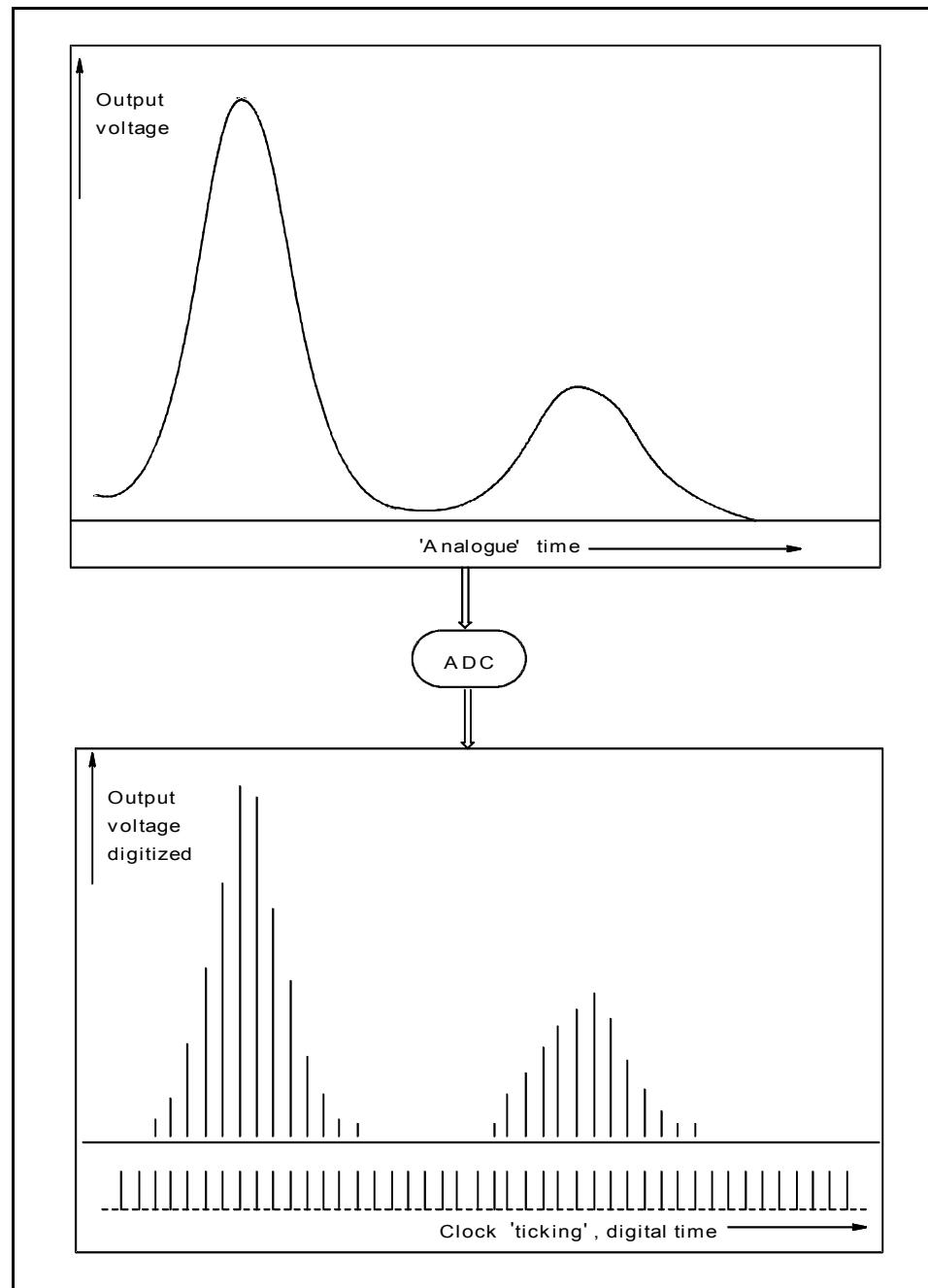


Figure 2 An analogue-to-digital converter (ADC) converts a continuous signal into a series of digital pulses in which the voltage represents 'snapshots' of the analogue signal taken at regular time intervals.

With the artificial baseline the voltage reading between peaks is constant and the true peaks clearly rise up out of the baseline and then fall back below again (Figure 5). Note that the artificial baseline should not be set at such a high value that it covers most of the peaks because this would lead to false estimates of the peak heights. As a peak starts to form, the change in voltage is noted by the pre-processor which then collects the digitized data until the peak disappears (the voltage returns to its steady value). From the digital voltage readings at the known time intervals the pre-processor calculates the peak centroid (centre of gravity) as a measure of the position (m/z value) and the peak area (a measure of ion abundance) by simple mathematical algorithms illustrated in Figure 6. These two bits of information (peak area and arrival time), together with a 'flag' to enable the computer to keep track of the peak data, are sent to the computer memory which stores the information until the mass spectrometer stops scanning.

The spaces (time) between peaks is large compared with the peak width (measured as a time interval) and, if the above reduction in data did not take place, then something like one million bits of information would have to be stored per spectrum. In a typical spectrum there may be, say, 100 peaks and after passing through the pre-processor these correspond to only 300 pieces of information which are much easier to deal with than a million. Also, because the computer doesn't have to collect data when there is only the steady baseline (between peaks), it has a lot of time in computer terms to be doing other tasks. Thus, as each peak arrives (is detected) there is a rush of activity as the digitized signals are processed but between the peaks there are no data arriving and the computer can be programmed to use this time for other tasks (Figure 3).

Once the peaks have been collected and stored, the computer can be asked to work on the data to produce a mass spectrum and print it out or it can be asked to carry out other operations such as library searching, producing a mass chromatogram and making an accurate mass measurement on each peak. Many other examples of the use of computers to process mass data are to be found in these guides.

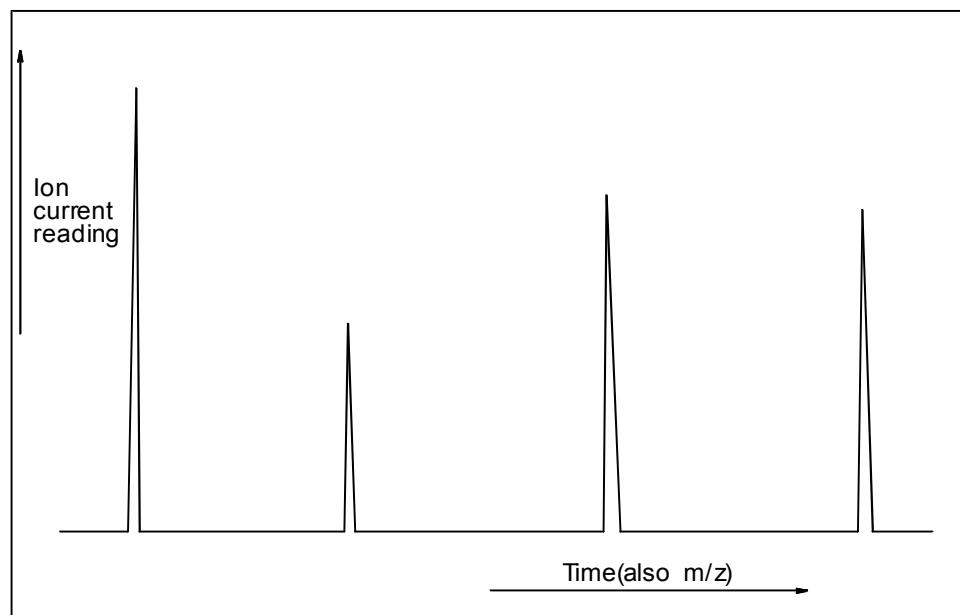


Figure 3 The voltage from the ion detector varies as ions are brought to a focus. As each m/z value is focused, a 'peak' ion current leads to the voltage change. Between the individual m/z values (between the peaks), no voltage change is observed because no ions arrive at the detector. The time between individual peaks is very much longer than the time representing peak width.

Instrument Control

(a) Peak Shape

In a well-tuned (adjusted) instrument the shape of a mass spectral peak is approximately triangular (Figure 7) but, in an instrument which is badly tuned, the peak will appear misshapen (Figure 7). Usually, the cause of the skewing of the peak arises from incorrectly adjusted voltages in the various electric or magnetic lenses used to align the ions in flight so that they are focused at a collector (see Guides on 'Ion Optics' for sector and quadrupole mass spectrometers). The maladjusted voltages may cause some ions to have too much or too little momentum or may even rotate the beam somewhat so that it doesn't pass truly through the defining slits. The misshapen peak can be detected by the computer. The digital voltage samples obtained across the peak (as described above) are compared with those expected for a 'perfectly' shaped peak. Then, the computer is programmed to send out signals to various of the ion optic lenses in turn to increase or decrease the voltages on them so as to correct the astigmatism in the ion beam. Each time an adjustment is made, the computer checks the new peak shape. This process continues until the shape is right. The process has been completely automated and, in the latest mass spectrometers, getting the peak shape correct is all part of an automatic setting up process.

(b) Voltage Checking

A mass spectrometer has a number of instruments attached which give information on its functioning. For example, the vacuum system is monitored by an ion gauge which gives out an electrical signal proportional to the vacuum being achieved. Thus, the computer can check this voltage routinely and, if it finds a reading indicating a fault, it can draw it to the attention of the operator or, if the fault is serious, the computer can shut down the electronic components of the spectrometer to prevent them from being damaged. If, for instance, the pressure in an electron ionisation source rises too high the filaments will burn out; early detection of the imminence of such a state is invaluable in time and repair. This sort of checking is arranged to be done routinely and, because of the speed of a computer, it can be carried out on a very short cycle time (e.g., once a second). Similarly, other parts of a mass spectrometer are routinely checked on a cyclical basis. For many faults, programming has reached a stage where the computer, having detected a fault, can diagnose it and report it to the operator.

Clock 'ticks' (sec)	Detector output (volts)
.	.
5.00001	3.5
5.00002	5.0
5.00003	15.1
5.00004	6.2
5.00005	4.3
5.00006	3.5
.	.
.	.
5.21001	3.5
5.21002	3.5
5.21003	3.5
5.21004	3.5
5.21005	3.5
.	.
.	.

Figure 4 A typical flow of digital data for a small part of a mass spectrum. After 5 seconds of scanning an ion peak appears, the digital voltages being read off from the analogue signal at intervals of 0.0001 seconds. At other times only a steady baseline voltage reading is observed.

(c) Different Scanning Modes

The primary objective of data processing is to obtain the ‘routine’ mass spectrum, viz., to produce a chart showing the number of ions (ion current or ion abundance) at each m/z value within a given pre-set range of m/z values. To this end, the computer controls electrical or magnetic fields so that scanning of one or the other starts at some predetermined value and changes uniformly until a second predetermined point is reached. The changing fields allow ions of steadily increasing (or decreasing) m/z values to reach the ion detector where they are recorded, also by the computer. However, there are more complicated scanning modes required for ‘metastable’ ion work and for MS/MS. The actual nature of these modes is set out in the corresponding Guides. Here, it is noted that the scans are electrically and/or magnetically quite complicated, requiring the fields to be scanned together in predetermined fashioned. Thus, a B^2/E scan needs the square of a magnetic field strength to be varied with a first-order change in the electric field so that the ratio of B^2 to E remains constant as a mass spectrum is obtained. Since the computer can monitor the field strengths (item b above) it is able to effect the required variation whilst, at the same time, acquiring the incoming mass data. This dual working function could be done by having transputers working in parallel (one for acquiring and processing data and the other for checking and controlling field strengths). Alternatively, a single microprocessor deals with both functions ‘at the same time’ but actually by switching extremely quickly from one function to the other iteratively.

Manipulation of Mass Spectral Data

Apart from the actual acquisition of the mass spectrum and its subsequent display or print-out, the raw mass spectral data may be processed in other ways, many of which have been touched on in other guides in this series. Some of the more important aspects of this sort of manipulation of data are explained in more detail below.

(a) Library Searching

Most mass spectrometers for analytical work have access to a large ‘library’ of mass spectra of known compounds. These libraries are in a form that can be read immediately by a computer, viz., the data corresponding to each spectrum have been compressed into digital form and stored permanently in memory. Each spectrum is stored as a list of m/z values for all peaks which are at least 5% of the height of the largest peak. To speed up searching, a much shorter version of the spectrum is normally examined (e.g. only one peak in say every 14 mass units).

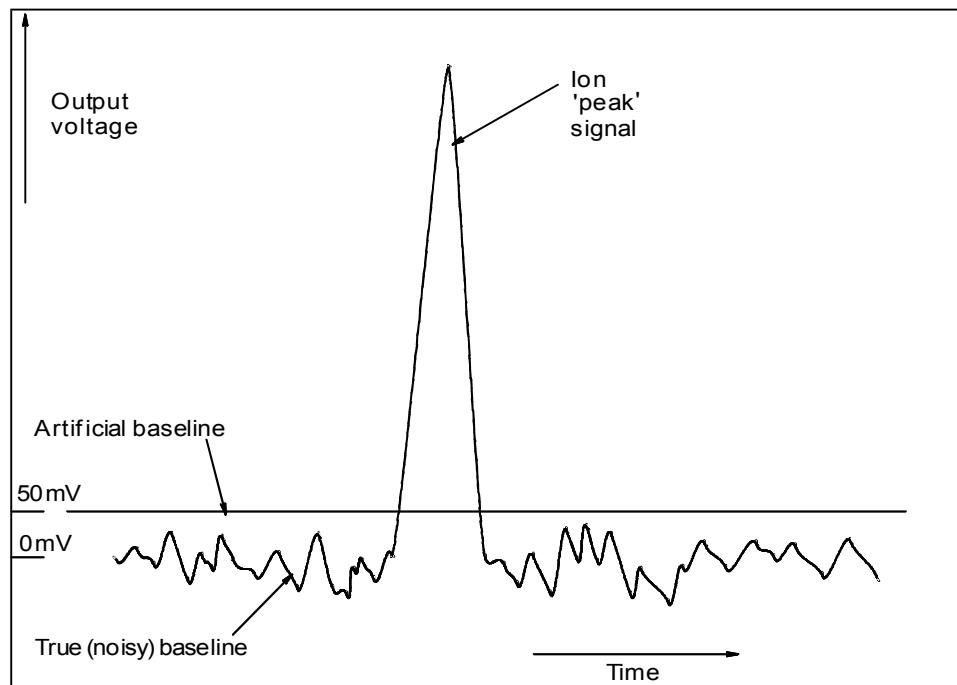


Figure 5 A true baseline output from an ion detector is electrically noisy and, if recorded as such, the noise would appear as a great many small (unwanted) peaks. By creating an artificial baseline at a voltage just above the noise, the small peaks are 'eliminated' and only the desired signal is recorded. It is important not to set the artificial baseline voltage too high or else too much of the required peak is eliminated also.

When a mass spectrum has been acquired by the spectrometer/computer system it is already in digital form as m/z values versus peak heights (ion abundances) and it is a simple matter for the computer to compare each spectrum in the library with that of the unknown until it finds a match. The shortened search is carried out first and the best 'fits' or 'matches' between the unknown and spectra in the library are reported. This sort of searching of even 60-70,000 spectra takes only a few seconds, particularly if transputers are used, and saves the operator a great deal of time. Even a partial match can be valuable because, although the required structure may not have been found in the library, it is more than likely that some of the library compounds will have structural pieces which can be recognised from a partial fit and so provide information on at least part of the structure of the unknown.

(b) Presenting Data in Different Ways

Once mass spectral data have been transformed into a set of m/z values together with ion abundances and put into memory, the computer can be programmed to manipulate the information in different ways. For example, instead of a straightforward mass spectrum, it can produce a normalized one in which all the peak heights have been recalculated with respect to the largest one which is made equal to 100%. In GC/MS and LC/MS operations, the computer will present a Total Ion Current Chromatogram or a Mass Chromatogram (Figure 8) or it can be used for Selected Ion Monitoring (see relevant Guides). All of these sorts of computer operations are done independently of the acquisition of data and of spectrometer control functions and are done through specially written software programmes acting on the acquired and pre-processed data. In fact, once the data have been processed into m/z values and ion abundances, any of a large number of possibilities can be carried out if the relevant programme is available. Manufacturers usually supply a suite of such programmes and, depending on the way the computer/transputer has been set up, these additional software programmes may be run only when the computer is not acquiring data and controlling the spectrometer (foreground working) or they may be run at the same time as data acquisition (foreground/background working). The latter mode is more powerful but requires enhanced computer processor capacity, often provided by use of one or more transputers.

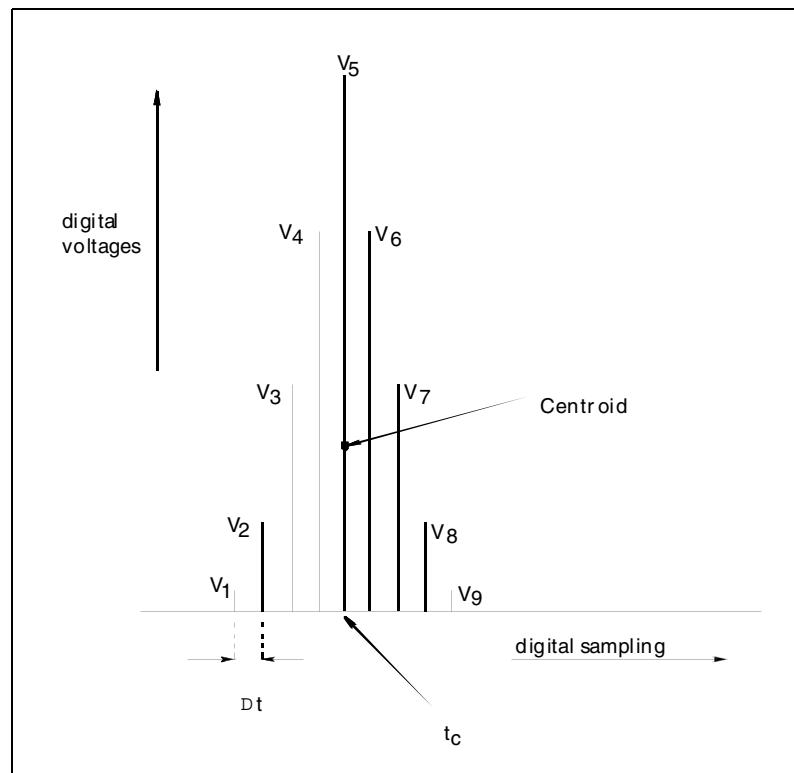


Figure 6 If digital voltage readings ($V_1 - V_9$) are taken at time intervals (Δt ; 0.00001 seconds in the example of Figure 4) then the area of the true peak (dotted) can be mathematically closely approximated to give ion abundance and similarly the time (t_c) to the centre of gravity (centroid) of the peak can be determined, thereby giving the m/z value.

(c) Accurate Mass Measurement

Assuming that the mass spectrometer has sufficient mass resolution, the computer can be used to prepare accurate mass data on the m/z values from an unknown substance. To do this, it is necessary for the system to acquire the mass spectrum of a known 'reference' substance for which accurate masses for its ions are already known and the computer has a table of these masses. The computer is programmed first to inspect the newly acquired data from the reference compound in comparison with its stored 'reference' spectrum; if all is well, the system goes on to acquire data from the unknown substance. By comparison and interpolation techniques using the (known) reference spectrum (Figure 9), the m/z values from the spectrum of the unknown are calculated accurately (see Accurate Mass Guide). Interestingly, if the original comparison of the spectrum from the reference compound with stored m/z data for that compound reveal discrepancies, the stored reference data are updated before the computer goes on to acquire data from the unknown compound. In this mode the computer is not used simply to acquire and manipulate data but is also used to take decisions regarding the accuracy of the figures it holds about the known reference; any discrepancy leads the computer to refresh its reference table.

Conclusion

Computers, often combined with transputers, are used for three main functions when connected to a mass spectrometer. Foremost are the requirements for acquisition and pre-processing of basic data and control of the scanning operations of the instrument. By use of a suite of additional software programmes, the pre-processed data can be manipulated in a wide variety of ways depending on what is required - a mass spectrum or a total ion chromatogram for example.

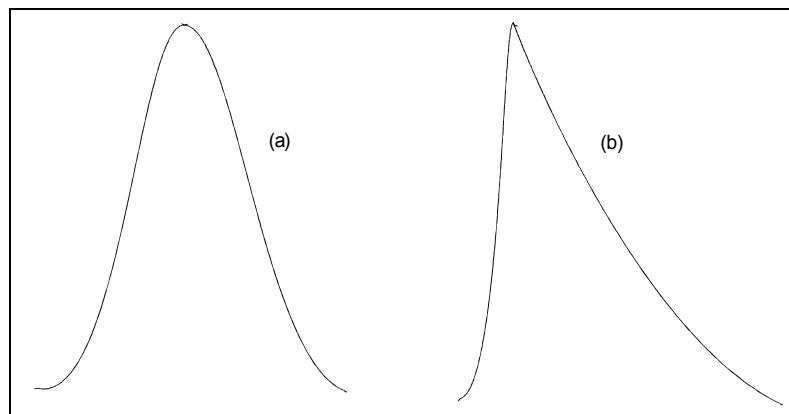


Figure 7

(a) In a well-tuned instrument, the ion beam has only a small spread of translational energies causing the ions to arrive at the collector evenly and closely grouped around an average time of arrival. The resulting analogue signal (peak) is approximately an isosceles triangle.

(b) In a poorly tuned instrument, the peak is distorted, with more ions on one side of the beam than the other. The mean arrival time in such an instance is no longer the same and leads to error in the recorded m/z value, particularly in accurate mass work.

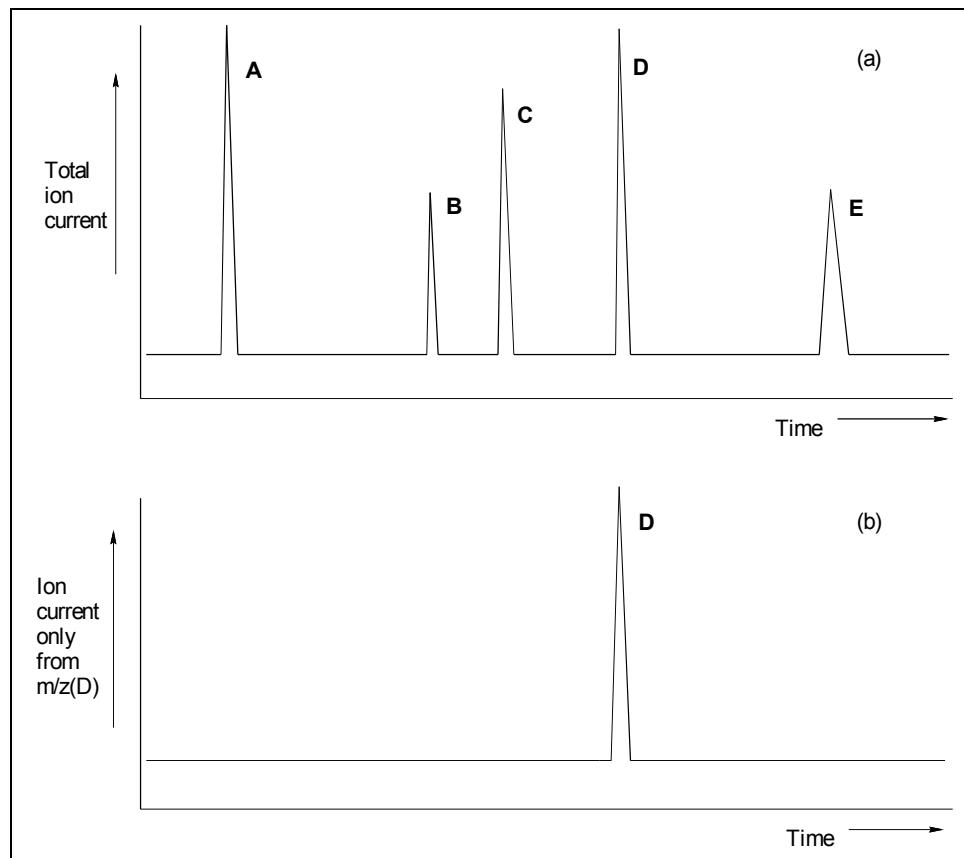


Figure 8 (a) The output from GC/MS or LC/MS instruments can be a total ion current chromatogram (TIC) showing the detection of all the substances (A - E) eluting from the chromatographic column or, (b) by setting the computer to ignore all ions except those characteristic of, say compound D, only that one peak will appear in the resulting mass chromatogram.

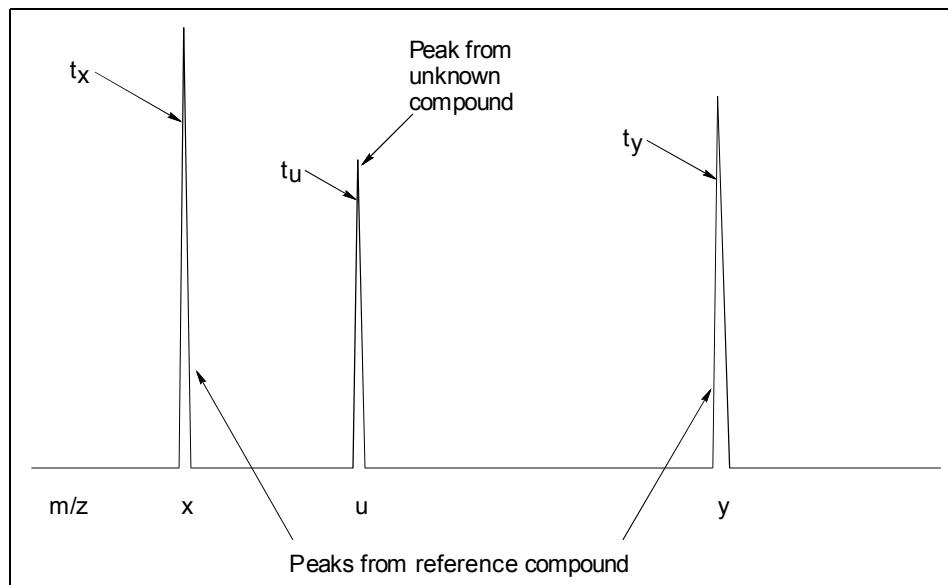


Figure 9 If the m/z values (x, y) for two peaks in a reference compound are known, the m/z value (u) for an unknown lying between them can be calculated accurately by interpolation. The m/z values are actually of course peak arrival times at the ion collector (t_x, t_u, t_y) and so,
 $m/z(u) = m/z(x) + \{(t_x - t_u)/(t_x - t_y)\} \{m/z(x) - m/z(y)\}$.

All the data on the right of this equation are known and so $m/z(u)$ is easily calculated.

Back to Basics Section B: Interfaces and Ionisation Techniques

CHAPTER B8

LASERS AND OTHER LIGHT SOURCES

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Quick Guide

- Lasers are sources of highly collimated, coherent and intense beams of light, which may be obtained commercially from the ultraviolet into the far infrared.
- The small cross-sectional area covered by a laser light beam coupled with the energy density in the beam leads to power levels reaching from milliwatts to many hundreds of kilowatts per square metre.
- The weaker lasers are used in such systems as CD players and recorders and in communications and distance measuring devices. The more intense laser beams are used for welding and cutting of metals, cloth, skin and so on and have even been examined as means of inducing thermonuclear fusion reactions.
- The high intensity of a laser beam (many photons per unit area in unit time) means that, on irradiation, each molecule of a photon-absorbing substance may absorb one, two, three or more photons in rapid succession before the molecule can relax and return to its ground state.
- This behaviour is unlike the more usual experience of light sources that are capable only of providing one photon per absorbing event in the time taken for the absorbing molecule to return to its ground state.
- The multiphoton absorption leads to electrons in the irradiated molecules being raised to highly excited states. The excitation may be such that the sample is ionized directly to form a plasma. This is the basis of laser desorption ionization (LDI). Generally, there is only a relatively small excess of ions formed in this way and assistance from a matrix (MALDI) or secondary ionization (plasma torch) is necessary in order to obtain a better yield of charged species.
- With somewhat greater power levels, the laser beam is capable of putting so much energy into a substance in a very short space of time that the substance rapidly expands and volatilizes. The resulting explosive shock wave travels through the sample, subjecting it to high temperatures and pressures for short times. This is ablation.

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- The ablated vapours constitute an aerosol, which may be examined using a secondary ionization source. Thus, passing the aerosol into a plasma torch provides excellent means of ionization and, by such methods, isotope patterns or ratios are readily measurable from otherwise intractable materials, such as bone or ceramics.
- If the laser is used with a matrix, which “dissolves” the sample as a solid solution, the rapid expansion of the matrix in the laser beam co-volatilizes the entrained sample. Proton transfer from the matrix occurs to give protonated molecular ions of the sample. Normally thermally unstable, polar biomolecules such as proteins give good yields of protonated ions. This is the basis of matrix assisted laser desorption ionization (MALDI).
- The three techniques of laser desorption ionization, laser ablation with secondary ionization and matrix assisted laser desorption are all used for mass spectrometry of a wide variety of substances from rock, ceramics and bone to proteins, peptides and oligonucleotides.

Summary

The large energy density in a tightly collimated laser beam is particularly useful for injecting large amounts of energy into sample molecules in a very short space of time. After absorption of this energy, the molecules relax by converting it into rotational, vibrational and kinetic energy within a few picoseconds. Direct ionization may result from the absorption but the level of ionization is usually assisted by having a secondary ion source (as with plasma torches used in isotope work) or by proton transfer from a matrix to give quasimolecular ions of thermally sensitive molecules such as peptides or proteins.

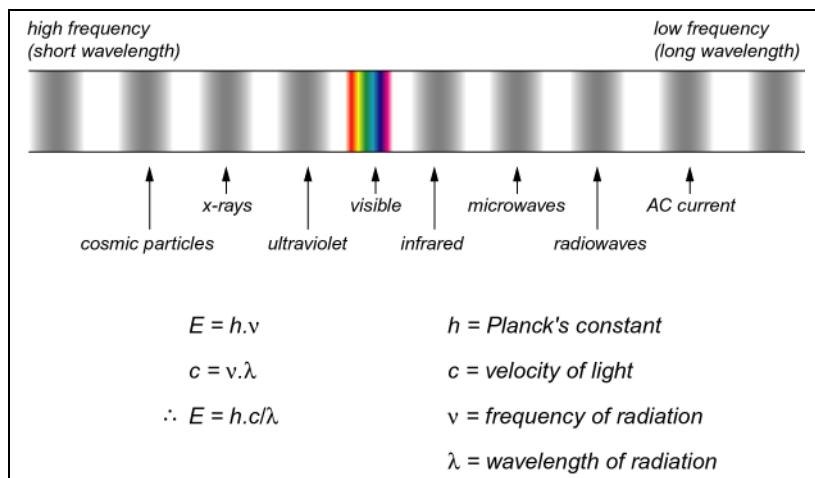


Figure 1 The electromagnetic spectrum ranges from high energy cosmic rays (high frequency, v) to low energy alternating current (low frequency, v). Different parts of the spectrum need different detection systems. The human eye detects light (blue to red) and the human body can detect heat (infrared) but humans can detect no other sections of the electromagnetic spectrum without the aid of instruments. Frequency and wavelength are inversely related so that high frequencies imply short wavelengths and vice versa. The above equations relate energy, frequency, wavelength and Planck's constant.

LASERS AND OTHER LIGHT SOURCES

Introduction

Before discussing light sources generally, it may be useful to consider some basic characteristics of light. Visible light composes a very small section of the electromagnetic energy spectrum, which ranges approximately from cosmic ‘rays’ to radio waves (Figure 1). The wavelengths of electromagnetic energies are related to the velocity of light (c) through the formula, $c = v\lambda$, in which λ is the wavelength and v is the frequency of the radiation. The highest energies are associated with highest frequencies and the lowest energies with lowest frequencies (Figure 1). Wavelengths follow the inverse order.

The smallest unit (“packet”) of electromagnetic energy (a photon) is related to frequency by the formula, $E = h.v$, in which E is the energy and h is Planck’s constant. Alternatively, the relation may be written, $E = h.c/\lambda$. Frequency (v) is a number with “units” of cycles per second (cps; the number of times a wavefront passes a given point in unit time; s^{-1}) and is given the name Hertz (Hz). Planck’s constant is a fundamental number, measured in J.s or erg.s.

Emission of light from various devices has been known for millions of years (sun, lightning, fireflies). Fires, oil lamps, candles, gas lamps and similar all use chemical reactions (combustion in air) to make “hot” (active) atoms or molecules, which emit light on cooling. In more recent times, electrical production of light has become commonplace as in tungsten lamps, fluorescent lights and arc lamps. The tungsten filament lamp uses electrical resistive heating to make a wire glow “white” hot but fluorescent and arc lights use forms of electrical discharge, similar in principle to the natural phenomenon of lightning (see Back-to-Basics guide on *Coronas, Plasmas and Arcs*). Photodiodes convert electrical energy directly into light, without the need for heating or discharge. In the period 1950-1960, a new form of light emission was developed, for which the acronym LASER was used (Light Amplification by the Stimulated Emission of Radiation).

The amount of light emitted by a source is measured by its luminance or by its luminous intensity, which are defined in Figure 2. Intrinsic light emission relates to the amount of light emitted per unit area (luminance). Table 1 lists approximate luminances some common light sources.

Units of light

The units in which light is measured are summarised briefly here.

The energy emitted by a light source and the energy falling on an object are two of the most important measures of the amount of light. Since the energy of a photon varies according to its wavelength ($E = h.c/\lambda$), measurement of the numbers of photons emitted or received gives a measure of energy emitted or received. The standard for light sources is emission at 555 nm. At this wavelength, the energy of one photon is 3.6×10^{-19} J.

The standard unit of light measurement (the light flux) is the *lumen*, which is the amount of energy (power, *watts*) emitted or received (*joules per second*). At 555 nm, 1 lumen = 0.00147 watts = 0.00147 J.s⁻¹. This is sometimes called a "lightwatt". Since the energy for one photon at this wavelength is 3.6×10^{-19} J, then the number of photons represented by 1 lumen is approximately 4×10^{15} per second, radiated or received. Thus, the luminous flux (lumens, *I_m*) gives the power radiating from an object or the power received by an object.

The number of lumens indicates the total amount of power but gives no indication of the "density" of that power. This last measure is given by the luminous intensity:

$$\text{Luminous intensity} = \text{lumens}/\text{steradians} \quad (\text{candelas})$$

The intensity is the flux of light passing through a solid angle of 1 steradian (1 steradian = the angle subtended at the surface of a sphere, for which the area illuminated is the square of the radius). Thus, 1 steradian (sr) is 1 cm² at the centre of a sphere 1 cm in radius or it can be 1 m² at the centre of a sphere of radius 1 m, and so on. The luminous intensity is a measure of the power density being radiated and the unit is the *candela* (cd). For any one power setting (lumens), the greater the solid angle, the less the luminous intensity. A laser beam has an extremely small angle of divergence (10^{-8} sr) and therefore its luminous intensity is very high even though the actual power may be low.

Luminance is the luminous intensity divided by the area of emission of light (lumens/steradian/m²). This is the power density emitted per unit area.

The luminance relates to the luminous intensity radiated from an entire surface of a light source:

$$\text{Luminance} = \text{lumens}/\text{steradians}/\text{m}^2 = \text{luminous intensity}/\text{m}^2$$

If the amount of light is measured over an area of receiving surface, the energy falling on the surface is measured in lumens per unit area ("lux" or "phot"). Thus, the number of lux = lumens/m² and this measures the power received per square metre of surface (energy per second/per unit area) and phot = lumens/cm² and measures the light power received per square centimetre of surface.

An international "candle" = 1 lumen.

Some examples of approximate luminances of various light sources are given in Table I.

Figure 2

The common units of light intensity or power density of light emitted or received are as shown above. Care should be taken in comparing luminances. For example, Table I reveals that a tungsten filament lamp has about a tenth of the luminance of the sun but the area of the sun's emitting surface is massively greater than that of a filament lamp and therefore the luminous intensity of the sun is massively greater than the luminous intensity of the filament lamp.

Almost all of the oldest light sources give light, which covers a range of wavelengths and is not coherent, *viz.*, the light waves are not propagated in phase with each other. The development of the laser has provided light sources, which emit sharply monochromatic, coherent and intense radiation, ranging from the ultraviolet to the infrared. Apart from their use in research, lasers have found important applications in a large range of everyday devices from CD players to metal plate cutters and welders. It is not the intention of this guide to cover this huge range of applications. Instead, this article concentrates on principles and descriptions of the most commonly used, commercially available lasers. Table 2 indicates some of the powers obtainable with various types of laser.

Up until about the 1990's, visible light played little intrinsic part in the development of mainstream mass spectrometry for analysis but, more recently, lasers have become very important as ionisation and ablation sources, particularly for polar organic substances (MALDI) and intractable solids (isotope analysis) respectively.

Some characteristics of light as a waveform

(a) Wavelength

Light sources may emit photons over a wide range wavelengths, as for example with "electric" light bulbs, or at a single wavelength (lasers). Therefore, a light beam may consist of a single wavelength, multiple wavelengths and broad band radiation (Figure 3). Various devices (filters, interference devices, diffraction gratings and monochromators) are available for selecting particular wavelengths from broad band radiation so as to give selectively more monochromatic light.

(b) Intensity

The intensity of a light beam refers to the numbers of photons it contains, regarding as passing through a unit area (flux). The intensity is a reflection of the amplitude of a multiphoton waveform, which varies with the numbers of photons and whether or not the light is coherent or incoherent. The power emitted by a light source or received by an object (lightwatts) relates to the energy of each photon (Figure 1), the number of photons and the time for which light is emitted or received (Figure 2).

Table 1. Some approximate luminances of various light sources

Light source	Luminance (candelas/cm ²) ^a
Sun (noon)	160,000
Tungsten filament lamp	12,000
Fluorescent lamp	0.82
Mercury discharge lamp	970
Metal halogen bulb	810
Photoflash light	20,000

^aCandela (*cd*) = lumens (*lm*)/steradians (*sr*), as shown in Figure 2

Table 2 Some typical lasers and their power outputs

Lasing substance(s) ^a	Physical state	Laser wavelength (nm) ^b	Pulse length or continuous wave	Typical maximum power output (watts) ^c
Cr/alumina (ruby)	solid	694	nanoseconds	100 MW
Nd/glass	solid	1060	picoseconds	100 TW
Ga/As	solid	840	continuous	10 mW
Rh6G (dye)	liquid	600	femtoseconds	10 kW
He/Ne	gas	633	continuous	1 mW
CO ₂	gas	10600	continuous	200 W
Ar/F	gas	193	nanoseconds	10 MW

^aThese designations are popularly used to describe the basis of the laser but are not accurate descriptions of the chemical states

^bAs the wavelength moves into the infrared region, it is more common to change units from nanometers to micrometers (microns). For example, 10600 nm would be written as 10.6 μm.

^cThe maximum or peak power depends critically on the pulse length. An energy output of 1 Joule in one second is a power of 1 Watt but, if the 1 Joule is emitted in a picosecond, the power rises to $1/10^{-12}$, which is 10^{12} Watts = 1 TW.

(c)Coherence Consider two trains of light waves (Figure 4). If two photon waves are coherent, the waves are in phase and therefore the wave intensity (amplitude) is doubled at all points. If the waves are not coherent, the two waveforms are out of step (out of phase) and the amplitude does not equal the maximum attainable by coherent waves. It is even possible for light waves to overlap in such a way that there is no light whatsoever, viz., the waves cancel each other (they are totally out of step) and darkness results (interference). A coherent laser beam may be contrasted with an incoherent incandescent light beam. In the former, all of the maxima in the waveform occur together in a tightly bundled stream (a light beam), i.e., the power density is high; for incandescent light, all the waveforms overlap randomly and the power density is much lower (Figure 4).

(d)Directionality and divergence A light source may be relatively large and emit light in all directions and its luminous intensity is low (Figure 2). This is characteristic of an incandescent electric light bulb, for which mirrors, reflectors and lenses must be used to effect any focussing or collimating that is needed to provide a beam. In contrast, a laser source emits light as a very narrow beam having very little divergence. For the same power output, an incandescent lamp emits far fewer photons per unit volume in any one direction than does a laser. Unless focussed, light from an incandescent lamp is spread more or less uniformly over all space but, through its intrinsic nature, a laser source emits a light beam, which is tightly focussed in one particular direction.

(e)Conversion of energy into light Given suitable mechanisms, all forms of energy can be converted into light just so long as the initial energy is equal to or greater than the energy of photons of light required. For example, chemical reactions may emit light, usually when an intermediate or product of reaction is formed in an energetically excited state and needs to lose energy to return to its ground state. Mostly, this loss of energy is vibrational and rotational and occurs through collision with other molecules or the walls of the containment vessel. In other instances, the excess of energy may be lost by emission of photons of visible light (chemiluminescence). This phenomenon can be observed naturally in the light emitted by glow-worms or fireflies. Electrical energy may be converted into light either directly by heating a wire (filament lamp) or through its discharge through a gas (corona, plasma, arc, lightning, photoflash). The “shockwave” of an electron travelling at near the velocity of light in a particle accelerator or on nuclear disintegration

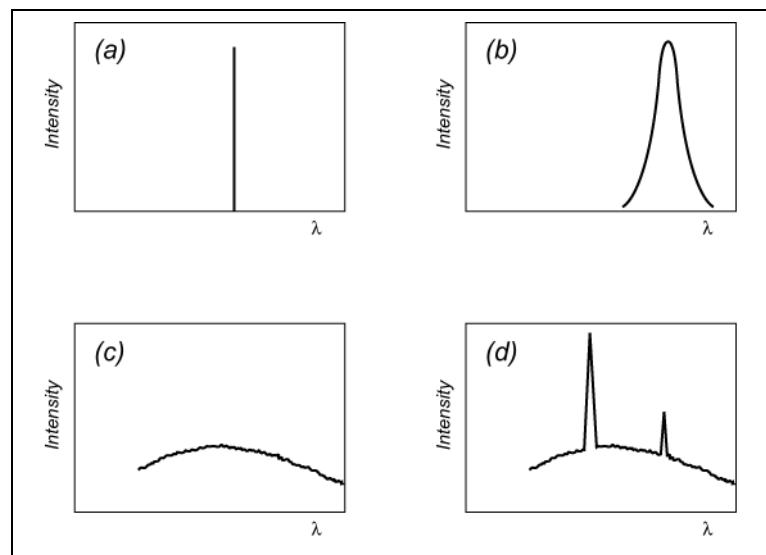
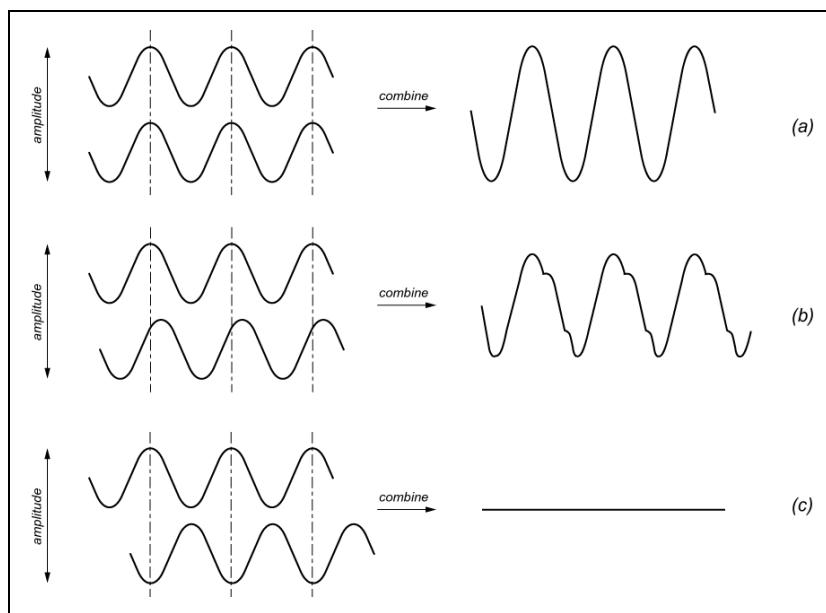


Figure 3 The diagrams depict some typical light outputs in relation to wavelength for various sources. (a) The sharp “line” (single wavelength) output from a laser or an atomic emission line. (b) The broader but still relatively sharp wavelengths obtained by using interference filters, monochromators and diffraction gratings or from molecular emission bands. (c) “Broadband” light output covering a wide range of wavelengths from such sources as the tungsten filament lamp. (d) A mixed output of a typical discharge lamp, having some intense narrow bands from atomic emission superimposed on broadband radiation from ion/electron recombination.

emits light (Cerenkov radiation). Atomic particles from the sun strike the earth's upper atmosphere, exciting nitrogen and oxygen so as to emit the beautiful lights of the aurorae.

Some non-laser light sources

- Incandescent lamps As mentioned briefly above, when an electric current is passed through a resistance wire it becomes hot and emits photons of heat and light. The higher the temperature of the wire, the more the proportion of light to heat but this form of lighting is inefficient in that much of the energy input is wasted as unwanted heat output. The emitted radiation is quantised but, because the quanta are separated by very small energy gaps, there is in effect a continuum of light stretching from the infrared to the ultraviolet region. The very high temperatures that are needed to cause thermal emission of ultraviolet light are not generally available but such emission is observed during welding for example. Short wavelength radiation emitted at high temperatures can be found in some stars, which are so hot as to emit a significant fraction of light towards the ultraviolet end of the spectrum and therefore appear blue rather than the common white, orange or red colours of stars like the sun. With everyday incandescent lamps, the emitted light tends to be yellowish because there is an excess of light emitted towards the red end of the spectrum (Figure 5).
- As described already, light from incandescent sources is emitted in all directions, is multichromatic (covers many wavelengths) and is incoherent. It is possible to select a single wavelength or even in a narrow band of wavelengths by using interference filters or diffraction gratings but only at the expense of rejecting most of the unwanted light. Focusing of selected light wavelengths is also inefficient through the need to use lenses and mirrors. Thus, when specific wavelengths are required, incandescent lamps are very inefficient and, unless they are extremely powerful, the output at any selected wavelength is relatively weak. This is in marked contrast to laser light, which is produced as a tightly collimated, narrow beam of intense monochromatic radiation.
- Arc lamps The basic principles of coronas, plasmas and arcs are discussed in these Back-to-Basics guides. Essentially, light emitted from such sources arises from gases at low, medium or high pressures, which are excited by the passage of electrons. Gas atoms are electronically excited (gain energy) or are ionised through collision with electrons.

**Figure 4**

In (a), two photon waves combine to give a new waveform, which has the same appearance and frequency as the initial separate waves. The photons are said to be coherent and the amplitude of the waves (light intensity) is simply doubled. In (b), the two photon waves are shown “out of step” in time (incoherent). Addition of the two waveforms does not lead to a doubling of amplitude and the new waveform is more complex, being composed of a doubled overlapping frequency.

In (c), the two waveforms are completely out of step (out of phase) and completely cancel each other, producing darkness rather than light (an interference phenomenon).

Light is emitted when the excited atoms return to their ground state by ejection of photons or when ions recombine with electrons and return to the ground state. The light is characteristic of the gas used for the discharge and consists of a mixture of narrow and broad band emissions. The narrow bands arise from atomic emission lines and the wide bands from recombination of electrons and ions (Figure 3a). Corona, plasma and arc sources are produced under a range of pressure conditions and current flows. Their emissions stretch from the ultraviolet to the infrared region of the electromagnetic spectrum.

Arc lamps are more efficient than incandescent light sources and radiate from a small region of space so that the light is much easier to focus or collimate than is the light from a heated filament. Because of this and the possibility of obtaining an intense light beam, arc lamps are used for many purposes, as in photolithography or photocuring of polymers or for lighting theatres but they cannot match the laser for the amount of energy deposited into a small space in a short time.

Laser light sources

Absorption and emission of light

To understand the production of laser light, it is necessary to consider the interaction of light with matter. Quanta of light (photons) of wavelength λ have energy E given by expression (1), in which h is Planck's constant (6.63×10^{-34} J.s) and c is the velocity of light (3×10^8 m.s $^{-1}$).

$$E = h\nu = hc/\lambda \quad (1)$$

Thus, green light from near the middle of the electromagnetic spectrum (Figure 1) has a wavelength of about 500 nm. From equation (1), this corresponds to a frequency $\nu = 0.6 \times 10^{15}$ Hz and an energy per photon, $E = 3.98 \times 10^{-19}$ J. If an atom or molecule has a ground state (A^0) and a suitable excited state (A^*) separated by an energy gap of 3.98×10^{-19} J then a photon of green light may be absorbed (Figure 6). The molecule is then in an excited state. Such atoms or molecules exist in the excited state for lifetimes, which vary with the structures involved but are generally of the order 10^{-13} to 10^{-8} seconds. After this time, the excited molecule returns to the ground state (A^0) by a variety of mechanisms. Sometimes this energy loss occurs simply by emission of a photon of the same or similar energy (wavelength) to that which excited it in the first place (Figure 6). Apart from this "normal" emission, ejection of a photon may be induced (stimulated) if the atom in its excited state is irradiated by a second photon.

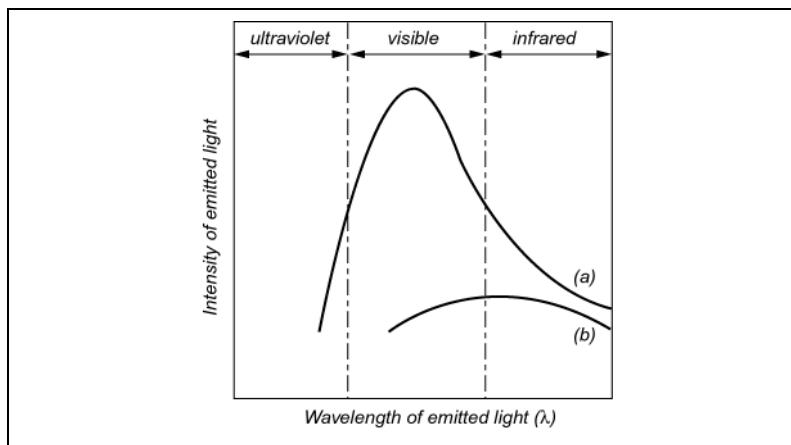


Figure 5 Curve (a) represents the intensity (number of photons) of light emitted from a hot incandescent object at about 5000 °C plotted against wavelength. The emission covers all visible wavelengths but not evenly and appears “white”. Curve (b) represents the radiation from a hot body at about 2000 °C (typical of an incandescent filament lamp). More light is emitted towards the red end of the visible spectrum and the light appears yellowish (there is less blue light). Compared with the amount of heat energy emitted, there is only a small proportion of light energy.

A major difference between “normal” emission and stimulated emission lies in the number of photons involved. For normal emission, a ground state atom or molecule first absorbs a photon and moves into an excited state and, some time later, emits a photon to return to the ground state (one photon absorbed, one photon emitted). For stimulated emission, a ground state molecule absorbs a photon and again enters an excited state. Before it can decay to the ground state, it interacts with another photon leading to two photons being emitted together (one photon in, two photons out). The two emerging photons travel in the same direction, are in phase (coherent) and have identical wavelengths. This stimulated emission can lead to a cascade effect in that one initial photon becomes two and then these become four, then eight and so on as they stimulate emission from other atoms or molecules in the excited state (A^*). This cascade of emerging photons, all travelling in the same direction, all in phase and all of the same wavelength, is the basis of laser light emission, whereby one photon event is amplified. Before this stimulated emission can become effective in production of a laser beam there are other requirements, which are considered next.

Populations of ground and excited states

The Boltzmann equation (2) shows that, under equilibrium conditions, the ratio between the numbers (n) of ground state molecules (A^0) and those in an excited state (A^*) depends on the energy gap E between the states, the Boltzmann constant k ($1.38 \times 10^{-23} \text{ J.K}^{-1}$) and the absolute temperature $T(\text{K})$.

$$n(A^*)/n(A^0) = \exp(-E/kT) \quad (2)$$

For the energy of excitation discussed above ($3.98 \times 10^{-19} \text{ J}$) and a temperature of 20°C (293 K), the ratio of excited states to ground states is, $n(A^*)/n(A^0) = 10^{-36.7}$. In other words, here is just one excited state atom or molecule for every $5,000,000,000,000,000,000,000,000,000,000,000,000,000,000$ molecules in the ground state! Thus, the chances of observing natural or stimulated emission are vanishingly small because there are almost no atoms or molecules in the excited state. For every photon entering such an assembly of molecules there are billions of chances of it being absorbed and only one of it inducing emission. Any photons so emitted would meet only ground state molecules and not another excited state atom or molecule required to start a cascade. Thus, an incipient cascade would stop before it could develop.

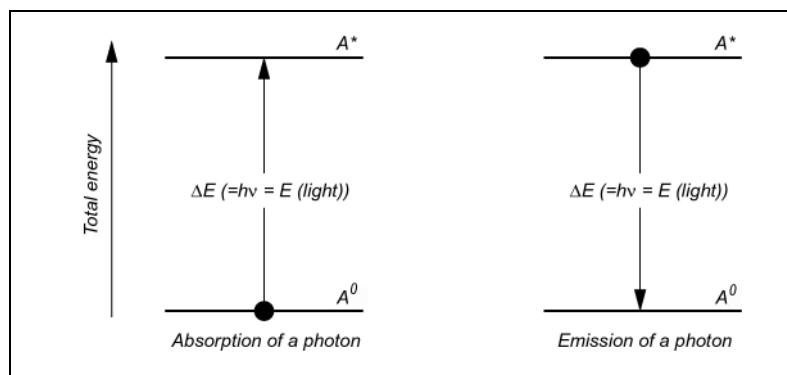


Figure 6 If an atom or molecule (A) in its ground state (A^0) has a more excited state (A^* ; higher energy) then the energy separation is ΔE . If a photon of light has energy $E(\text{light})$, which equals ΔE then the photon may be absorbed by A. On absorption, the atom moves from energy state (A^0) to the higher energy state (A^*) and $\Delta E = E(\text{light}) = h.v = h.c/\lambda$. Thus, if λ is the wavelength of light that corresponds to ΔE , the light can be absorbed. The state (A^*) may emit a photon of energy $E = h.v$ to return to the ground state (A^0).

If the temperature were raised, more molecules would attain the excited state but, even at 50,000 °C, there would be only one excited state atom for every two ground state atoms and stimulated emission would not produce a large cascade effect. To reach the excess of stimulated emissions needed to build a large cascade (lasing), the “population” of excited state molecules must be made to exceed that of the ground state, preferably at normal ambient temperatures. This situation of an excess of excited state over ground state molecules is called a population inversion in order to contrast it with normal ground state conditions.

Maintaining a population inversion

If the excited state has a very short lifetime, there becomes little chance of building up a population excess in the excited state because absorption is followed almost immediately by emission. This is typical for a simple two-level system of ground and excited states, as shown in Figure 6. To build up a population of excited state molecules, it is necessary that the excited state should have a sufficiently long lifetime before it returns to the ground state naturally. The easiest way of achieving this occurs when the initial excited state (A^*) decays to another excited state of lower energy (A'^*), which has a relatively long lifetime. This occurs frequently by spin inversion of an electron, as shown below and is the basis of a three-level system (two upper and one ground state; Figure 7).

If all spins ($\pm 1/2$) in an atom or molecule are paired (equal numbers of spin +1/2 and -1/2), the total spin must be zero and that state is described as a singlet (total spin, $S = 0$ and the state is described by the term, $2S+1 = 1$). When a singlet ground state atom or molecule absorbs a photon, a valence electron of spin 1/2 moves to a higher energy level but maintains the same spin of 1/2 and therefore the total spin remains zero; this excited state is also a singlet. Therefore, absorption of a photon is said to give a singlet to singlet transition. Emission of a photon to return to the ground state is “allowed”, giving a lifetime for the excited state of only a few picoseconds to nanoseconds. However, if the electron in the higher energy state can invert its spin, the system becomes a triplet (S now equals 1 because all spins are paired except for the two in the same spin state and therefore, $2S+1 = 3$). Return to the ground state is now not easy (it becomes a “forbidden” transition). In these circumstances, the lifetime of the excited state increases markedly to microseconds or milliseconds as the system has to find some other way of returning to the ground state. If sufficiently long, this increased lifetime allows a

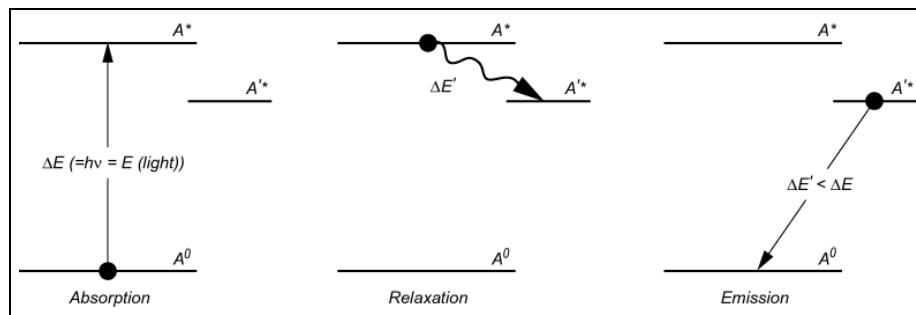


Figure 7 In a typical three-level system, initial absorption of light changes a ground state atom (A^0) to an excited state (A^*), as discussed in Figure 5. However, in the three-level system, the excited state atom or molecule can lose energy by collision or electronic state crossing to reach a more stable excited state (A'^*). Emission from this state gives photons of lower energy than the ones that led to the initial absorption ($\Delta E = h.v$, $\Delta E'' = \Delta E - \Delta E'$).

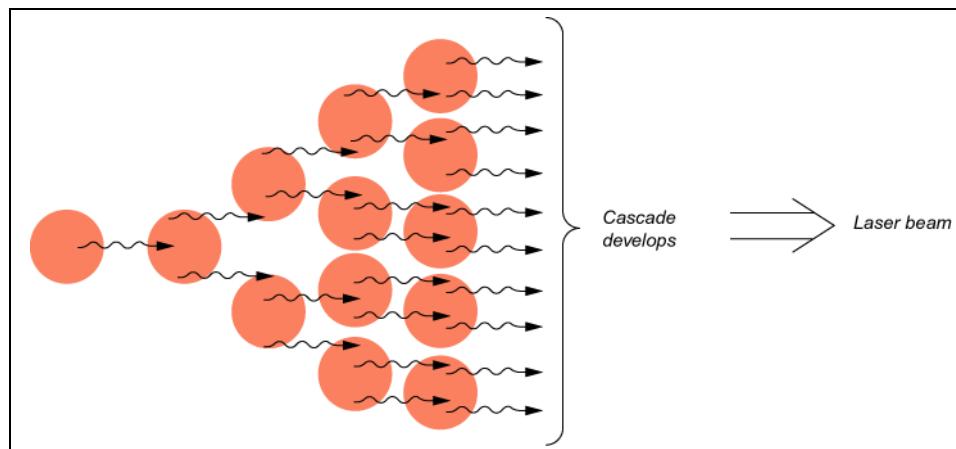


Figure 8 Interaction of an excited state atom (A'^*) with a photon stimulates the emission of another photon so that two coherent photons leave the interaction site. Each of these two photons interacts with two other excited state molecules and stimulates emission of two more photons, giving four photons in all. A cascade builds up, amplifying the first event. Within a few nanoseconds, a laser beam develops. Note that the cascade is unusual in that all of the photons travel coherently in the same direction. This is the reason for the very small divergence from parallelism found in laser beams.

high population of atoms or molecules in the excited state to build up rapidly. The population inversion needed for lasing can be achieved in various ways (Figure 7).

Lasing (emission of laser light)

Once a large inverted population of excited state atoms or molecules has been created, stimulated emission will occur readily. If one excited state molecule emits a photon naturally, there will be a very high probability that this photon will interact with another excited state molecule before it has travelled very far. This first interaction leads to stimulated emission of two identical photons. Before proceeding more than a few Ångstroms, each of these two photons interacts with other excited state molecules to give a total of four photons and so on; a cascade begins. Since the velocity of light is $3 \times 10^8 \text{ m.s}^{-1}$ then, within a few nanoseconds, a cascade of photons is produced, in which millions of photons are emitted from the over-populated excited state and make up an emerging laser beam (Figure 8).

Excited state levels

It was shown above that the normal two-level system (ground to excited state) will not produce lasing but that a three-level system (ground to excited state to second excited state) can enable lasing. Some laser systems utilize four or even five level systems but all need at least one of the excited state energy levels to have a relatively long lifetime, so as to build up an inverted population.

Pulsing and continuous wave

The timing of the emission is clearly dependent on the system in use. For example, if pumping is relatively slow and stimulated emission is fast then the emergent beam of laser light will appear as a short pulse (subsequent lasing must await sufficient population inversion). This behaviour is characteristic of certain types of "pulsed" lasers. Alternatively, if emission and population inversion ("pumping") are more or less in time with each other then the laser light appears more or less continuously, which is characteristic of "continuous wave" lasers.

Pumping (amplification)

Once laser emission has been generated, the excited state is depleted and must be repopulated before lasing can again take place. Excited state molecules may be generated by a variety of methods but these need to be repeated over and over so as to maintain the population inversion. This repeated replenishment is commonly known as "pumping". Thus, where a flash of light is used to obtain a population

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inversion, the flash must be repeated as long as the lasing action is needed. In effect, the flashes of light “pump” photons into one or more relatively long-lived excited states.

Methods of pumping up the excited state include electric discharges in gases, injection of current for semiconductors, flash lamps for solid state lasers, chemical reactions and even the light from other lasers.

The laser cavity

Once a population inversion has been built up, any naturally emitted photon can initiate the lasing action. To improve the overall effect, the laser generator is normally enclosed within a “resonance cavity”, which has mirrors at each end (Figure 8). The purpose of the mirrors is to cause the lasing action to travel up and down the cavity (less loss of light to the walls of the cavity), increasing the cascade in one direction so that most of the inverted population is stimulated to emit in a very short space of time. However, this action may prevent the excited state population from building up to a large excess and so the amount (intensity) of laser light output will be small. There are ways of improving the population of excited state molecules, two of which are “Q-switching” and “Mode locking”.

Q-Switching

As shown in Figure 9, any one emitted photon inside a laser resonance cavity can begin a cascade of photons. If the cascade reflects back and forth between the mirrors of the resonance cavity, most of the inverted population will be stimulated to emit “laser” photons. If one of the mirrors is temporarily made non-reflective, the initial cascade can no longer “bounce” back and forth inside the cavity and the numbers of stimulated emissions will be quite small (there is little laser light) and there is time for the excited state population to build up. If, after a short time, the non-reflective mirror is made reflective again, there will be formed rapidly a large cascade of photons back and forth inside the cavity, causing all of the overpopulated excited states to emit coherent photons. A “giant” pulse of laser light is emitted from the cavity.

One of the ways of making a mirror temporarily non-reflective is to place in front of it a solution of a dye that has suitable absorption bands. As long as the dye absorbs photons, none can get through to the mirror to be reflected. However, at some stage, as the number of photons in the cavity builds up, the dye molecules are all raised to their excited state in a short time and the dye loses its capacity to absorb any more light. It is said to be “bleached”. At this stage, the laser photons can reach the mirror, which is reflective. The laser photons can now “bounce” back and forth very quickly between the two mirrors of the cavity, stimulating emission from the large

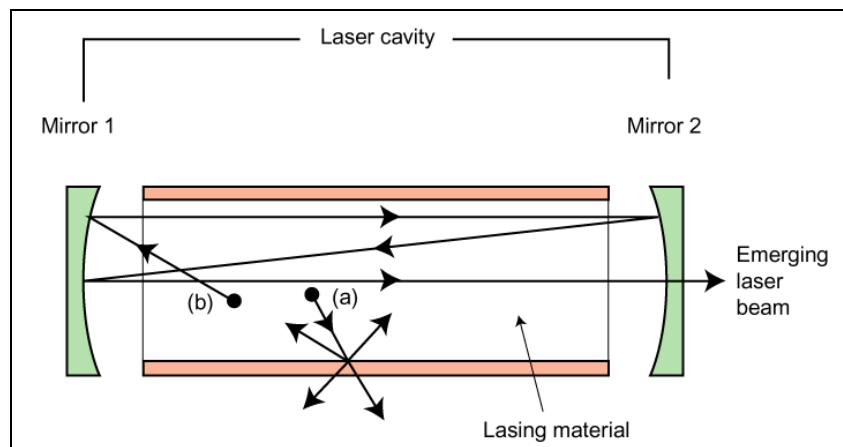


Figure 9 Once an inverted population has been built up, any one excited state atom or molecule may start a cascade of stimulated emissions. In (a), one such excited state atom has emitted a photon in the direction of the walls of the laser cavity. Although a cascade begins, when it reaches the walls, much of the light is scattered or travels through them and no laser beam emerges from the cavity. In (b), the excited atom emits a photon towards an end mirror (1); the developing cascade is focused onto the second mirror (2) and then back to the first and so on. In a very short time, a laser beam emerges from one of the mirrors, which is manufactured to be partly transparent instead of being totally reflecting.

inverted population that has had time to build during the time the dye was absorbing radiation. There is then a large pulse of intense coherent laser light lasting a few nanoseconds. After this event, most of the dye molecules return to the ground state and the mirror once more becomes non-reflective until just before the next giant pulse is emitted as the process repeats. This sort of Q-switching is said to be “passive” because it happens without any external intervention.

“Active” Q-switching occurs when laser light access to one of the mirrors in the cavity is controlled by an electro-optical cell, which works on the principle of affecting the passage of polarised light (see below; Kerr or Pockels cell). These last devices are able to turn on and off the transmission of light by using electric stimuli to alter the optical characteristics of the medium comprising the “cell”. In effect the cell acts as a very high speed shutter, controlled by a change in voltage. Application of a suitable electric potential prevents the passage of light and its removal allows it (or vice versa). Thus, by placing such a cell in front of one of the mirrors, laser light can be prevented from reaching the mirror until a large inverted population has built up inside the laser cavity. The cell is then switched to allow passage of photons, which pass up and down between the two mirrors and produce a giant pulse of laser light.

Mode locking

This is similar to the passive Q-switching except that the bleaching is effected by the differing “modes” of light. For any passage of light up and down the laser cavity, a “standing” wave must be built up, viz., if d is the distance between the mirrors then the frequencies of the standing waves are given by equation (3), in which n is the refractive index of the laser material and i is an integer (1,2,3...).

$$\nu = i \times (c/2d.n) \quad (3)$$

If $d.n$ “matches” ν , then standing waves are generated. If the match is not so good, fewer standing waves are formed. Thus, if as with passive Q-switching, there is an absorbing dye in front of one of the mirrors, the dye will absorb light as before. However, since frequencies ν that do not match $d.n$ very well are fewer than those that do match, this small number of frequencies is absorbed totally by the dye, leaving an excess of matching frequencies. Eventually, the dye is bleached by a the cascading effect of the selected modes (mode-locking; better matched frequencies). The time needed for a photon to make one trip up and down the cavity occurs as the dye becomes completely bleached. The resulting emergent laser pulse is very short and is highly monochromatic, since the low population of “unmatched”

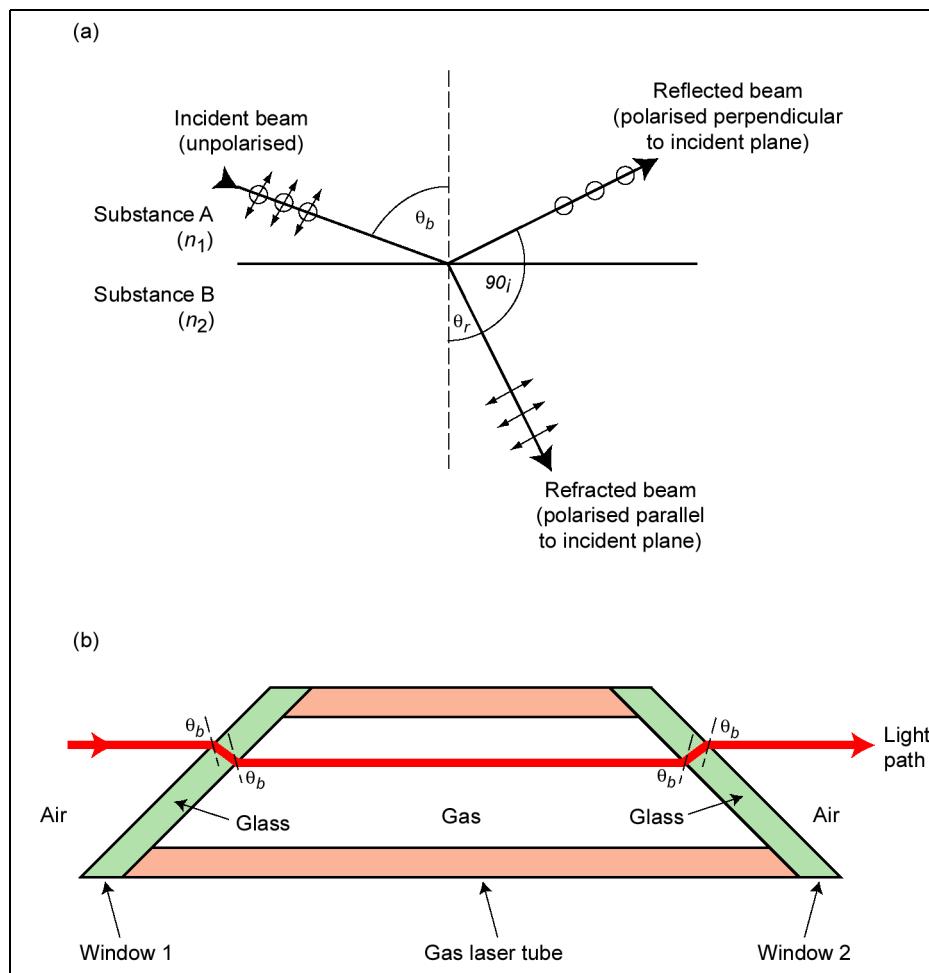


Figure 10 (a) A light beam incident at the surface separating two transparent substances (A,B) is shown to be partly refracted and partly reflected. When the incident beam is at the Brewster angle (θ_B), there is maximum transmission of the part of the beam polarised in a plane parallel to the plane of incidence (at right angles to the surface). The reflected part of the beam is polarised perpendicular to the plane of incidence (parallel to the surface). (b) For two windows at the ends of a gas laser tube, substance A is air and B is glass. The glass end plates are set at the Brewster angle for an air/glass interface so that a transmitted beam is passed through with maximum efficiency.

frequencies has been largely eliminated. The pulse also lasts only a very short time before the inverted population is lost and the dye returns to its unbleached state. The time taken for a photon to move up and down the cavity is the “pulse repetition frequency”, not to be confused with the frequency of the emerging laser light.

Brewster angle

When a beam of non-polarised light meets a surface separating two optically clear materials of differing refractive index (n_1, n_2) such as air and glass, part of the light is refracted (transmitted) and part is reflected at the surface. The refracted part of the beam is partly polarised in a plane at right angles to the surface (parallel to the plane of incidence; Figure 10) and the reflected part of the beam is polarised in the plane of the surface (perpendicular to the parallel component; Figure 8). As the angle of incidence of the incoming beam is decreased (Figure 10), less and less of the parallel component is reflected until, at the “Brewster angle” (θ_B), all of the parallel component is refracted (transmitted) and the reflected component is almost completely polarised in a direction parallel to the surface. At the Brewster angle, there is maximum transmission of light (a minimum of reflection), viz., the transmission is most efficient. There are two relationships that are characteristic of the Brewster angle. The first is that the refracted and reflected parts of the incident beam are at right angles to each other (equation 4) and the Brewster angle is related to refractive index by equation 5 (Figure 10a). For an air/glass interface, $\tan \theta_B = n$, the refractive index of glass. In a gas laser, the light must be reflected

$$\theta_B + \theta_r = \pi/2 \quad (4)$$

$$\tan \theta_B = n_2/n_1 \quad (5)$$

back and forth between mirrors and through the gas container hundreds of times. Each time the beam passes through the cavity it must pass through transparent windows at the ends of the gas container (Figure 10b) and it is clearly important that this transmission be as efficient as possible. By placing these windows in a plane set at the Brewster angle with respect to the light beam, maximum transmission is assured (minimum reflection). The above considerations regarding polarisation of refracted and reflected beams must be modified for non-transparent media such as metals, for which there is little polarisation of a reflected beam.

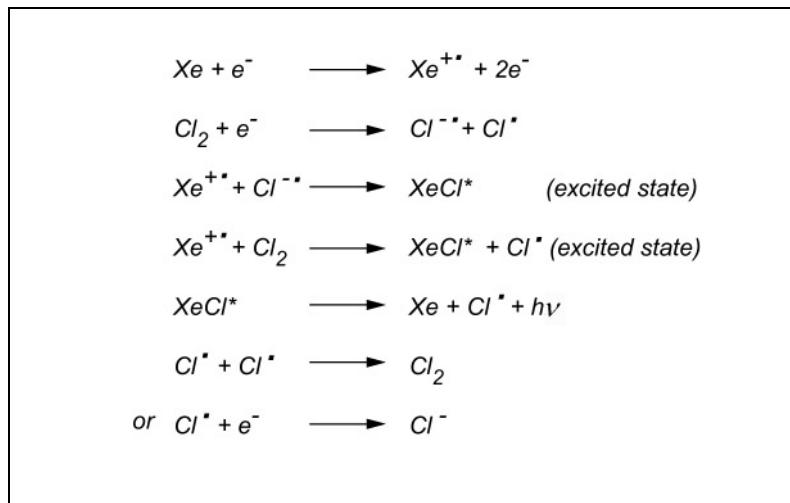


Figure 11 The reaction path shows how Xe and Cl_2 react with electrons initially to form Xe cations. These react with Cl_2 or Cl^- to give electronically excited state molecules $XeCl$, which emit light to return to ground state $XeCl$. The latter are not stable and immediately dissociate to give xenon and chlorine. In such gas lasers, translational motion of the excited state $XeCl$ gives rise to some Doppler shifting in the laser light so that the emission line is not quite so sharp as it is in solid state lasers.

Table 3. Wavelength of Laser Light Emitted from some Chemical Lasers

Chemical reactants	Excited state species	Laser wavelength (nm)
Argon, fluorine	ArF	193
Krypton, fluorine	KrF	248
Xenon, fluorine	XeF	351
Krypton, chlorine	KrCl	222
Xenon, chlorine	XeCl	308

**Methods of pumping
to obtain excited
states**

Only a few of the many methods that have been used are described here, viz., those for which commercial lasers are obtainable.

Gas state lasers(i) Molecular (chemical)
reaction

The principle of emission may be illustrated by means of an example. Suppose a mixture of two gases, xenon and chlorine, is contained within a laser cavity, which also has two metal electrodes. Under normal conditions, no compounds are formed between the two gases (for example, ground-state XeCl is not stable and cannot be isolated). If a strong electric field is applied across the two electrodes, some electrons are produced and these interact with the xenon so as to ionise it, producing more electrons (for more detail, see the Back-to-Basics guide on *Coronas, Plasmas and Arcs*). Some of the electrons attach themselves to chlorine (Figure 11). The net result is that, in a very short time, there is formed a population of positive xenon ions and negative chlorine ions. These combine to give XeCl in an electronically excited state (an excited state complex known as an exciplex, XeCl^{*}), in which the XeCl^{*} is stable for a short time. This provides a population inversion of excited state XeCl molecules. On stimulated emission, this electronically excited state returns to ground state XeCl by emission of light and, because it is not stable, the resulting ground state molecule dissociates into Xe and Cl (Figure 11).

By varying the types of gases inside the cavity, the wavelength of the laser emission may be varied (Table 3). These gas lasers are useful because the emitted light lies mostly in the ultraviolet region of the electromagnetic spectrum. Because of the translational motions of the atoms of gas, the emitted laser beam is not as highly collimated nor quite as monochromatic as is much laser light. In these lasers, excited state overpopulation is supplied continuously so that the laser light is also emitted continuously so that it is termed a “continuous wave” (cw) laser.

(ii) Molecular interaction

The examples of gas lasers described above involve the formation of chemical compounds in their excited states, produced by reaction between positive and negative ions. However, molecules may also interact in a formally non-bonding sense to give complexes of very short lifetimes, as when atoms or molecules collide with each other. If these “sticky” collisions take place with one of the molecules in an electronically excited state and the other in its ground state, then an

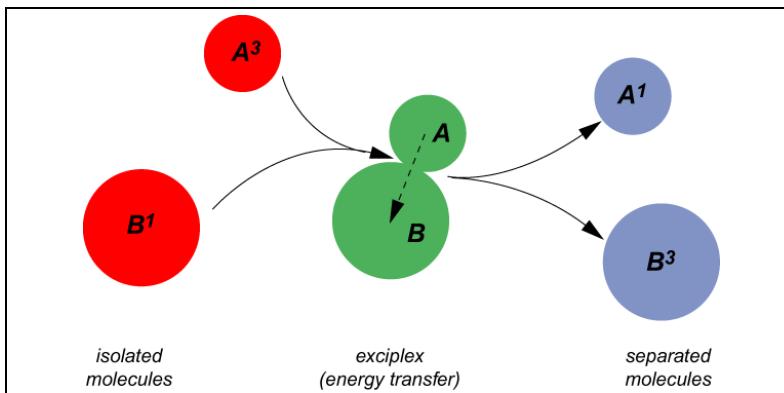


Figure 12 If a triplet state molecule A^3 meets a singlet state molecule (B^1), a short-lived complex may be formed (an exciplex). In the latter, the molecules exchange energy, A^3 returning to its singlet state (A^1) and B^1 being raised to its triplet state (B^3). If the new triplet state is relatively long-lived, it can serve to produce the population inversion needed for lasing, as in the He/Ne laser.

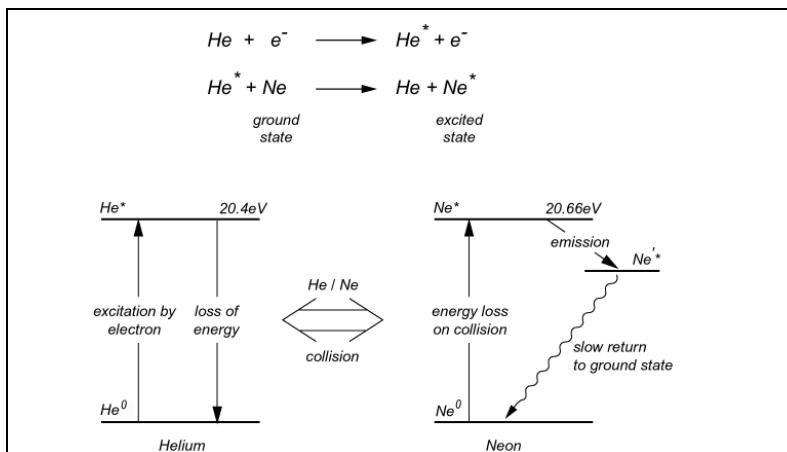


Figure 13 In a mixture of helium and neon, excited state helium atoms are formed on collision with electrons (electric discharge effect); the energy of this state is 20.4 eV above the ground state. When the He^* atoms collide with neon, energy exchange takes place to give excited state neon atoms (Ne^*) and ground state He. This excited state of neon is 20.66 eV above the ground state and is relatively long-lived so that an inverted population of Ne^* builds up until stimulated laser emission at 632.8 nm moves the Ne^* atoms to a lower energy state (Ne^{**}), which quickly returns to the ground state (Ne).

excited state complex (an exciplex) is formed, in which energy can be transferred from the excited state molecule to the ground state molecule. The process is illustrated in Figure 12.

This type of energy transfer is common and is used to promote some substances into excited states that are not easy to obtain by other means. For example, “normal” oxygen molecules exist in an electronic triplet state, which is relatively unreactive (just as well or you would not be reading this!). However, oxygen can be activated into its singlet state, which it is extremely reactive, causing the oxygen to chemically attack all kinds of substances. Direct irradiation of oxygen with light is very inefficient for effecting this triplet/singlet conversion, which is formally disallowed because an electron must invert its spin in the process. However, there are substances (sensitizers) that can be excited efficiently into triplet states. If one of these excited sensitizer molecules collides with a ground-state oxygen molecule then energy transfer occurs in the resulting exciplex, whereby the oxygen is translated into its triplet state with high efficiency and while the sensitizer returns to its ground state. Gas lasers can work on a similar principle, in particular the He/Ne laser (Figure 13).

If helium is part of a discharge gas, it is transformed into metastable ions of energy 20.4 eV. If, before it can return to the ground state, the excited atom collides with a neon atom, energy transfer occurs in such a way that the neon atom is excited into a 20.66 eV electronic state and the helium atom returns to the ground state. The small energy difference of 0.26 eV is taken up as a kinetic energy change in the exciplex. The excited state of the neon is relatively long-lived and an inverted population of this excited state builds up. Stimulated emission causes the production of laser light at a wavelength of 632.8 nm (Figure 13). The steady formation of helium atoms in excited states and the frequency of their collision with neon atoms produces a steady supply of inverted population so that the lasing action is continuous (cw).

Liquid state (dye) lasers

Dye lasers make use of delayed fluorescence to produce a laser beam. If suitable “dye” molecules are irradiated with light at their visible absorption wavelength, there is an interval of time until light is emitted as fluorescence. The wavelength of the fluorescent light is greater than the wavelength of the incident light (Figure 14). If the emitted light is passed back and forth between mirrors, then stimulated emission can occur to give laser light in the usual way. Rather than simply having two mirrors at the ends of the cavity, one mirror is replaced by a diffraction grating, which not only acts as a

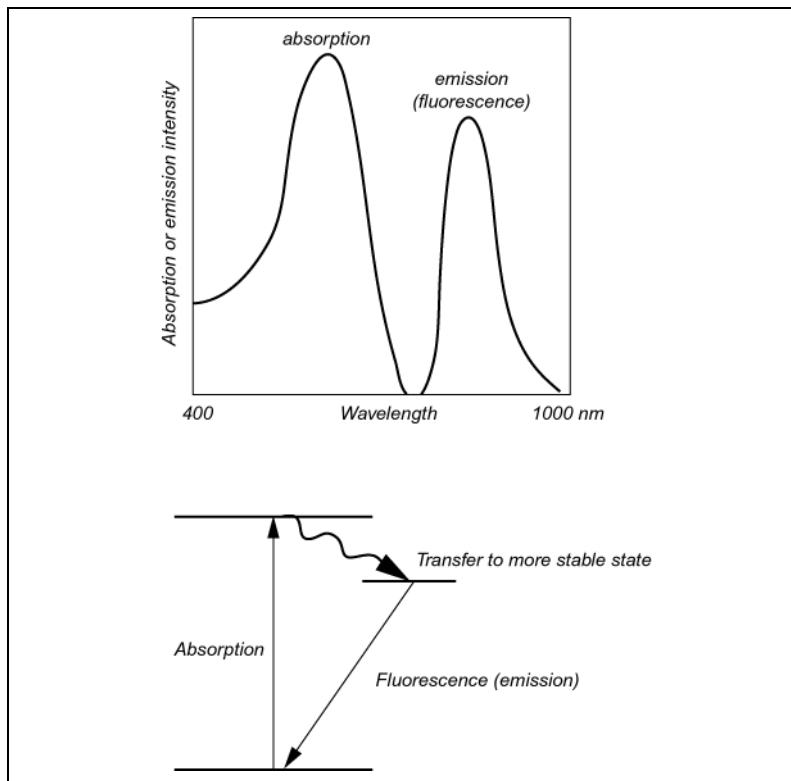


Figure 14 A dye molecule has one or more absorption bands in the visible region of the electromagnetic spectrum (approximately 350-700 nm). After absorbing photons, the electronically excited molecules transfer to a more stable (triplet) state, which eventually emits photons (fluoresces) at a longer wavelength (this composes a three-level system). The delay allows an inverted population to build up. Sometimes there are more than three levels. For example, the europium complex (Figure 15) has a four-level system.

mirror but also can be used to select a narrow range of wavelengths from the light falling on it (Figure 15). Therefore, after the first fluorescent photons (which cover a range of wavelengths) have been selected at the diffraction grating, the remaining reflected beam of photons covers only a very narrow range of wavelengths and passes through the dye solution, inducing stimulated emission of more photons of the same wavelength. Thus, the stimulated emission (emergent laser beam) covers a narrow range of wavelengths. To prevent complete bleaching of the dye during this process, the dye solution is arranged to flow through the cavity and then circulates back to a holding vessel before passing through the cavity again. To obtain high powers from the laser, the light used for pumping up the lasing levels to overpopulation, often comes from an argon laser.

The sorts of “dyes” that are used are materials, which absorb visible light and then emit visible fluorescent light. These are mostly rigid organic or metallo/organic materials, having extended π -systems, such as those shown in Figure 16. They are characterised by having a high quantum efficiency for converting incident light into fluorescent light. Rhodamine G is one of the most efficient of such fluorescent molecules.

Dye lasers are very useful in that their output can be tuned over a range of wavelengths. An organic dye (usually rhodamine G, one of the most highly fluorescent substances known) in solution, flows through a transparent cell.

Solid state lasers

(i) Ruby laser

Ruby (essentially alumina), owes its well-known colour to the presence of very small proportions of chromium ions (Cr^{3+}) distributed through it. “Ruby” lasers do not use natural rubies because of the imperfections they contain. Instead, synthetic single crystals of chromium ions (0.05%) in alumina are used. These lasers emit light at 694 nm, in the red end of the electromagnetic spectrum. The emergent laser light is produced in pulses of a few milliseconds and peak power may be tens of kilowatts.

Chromium ions produce the red colour of ruby because they absorb blue/green light from white light, leaving unabsorbed red light to be transmitted. For a ruby laser, white light is produced by a flash lamp situated alongside the ruby rod. When the chromium ions absorb the light, they are promoted to an electronically excited state, which rapidly loses some vibrational energy to cross into a more stable, longer-lived state and this provides the inverted population required

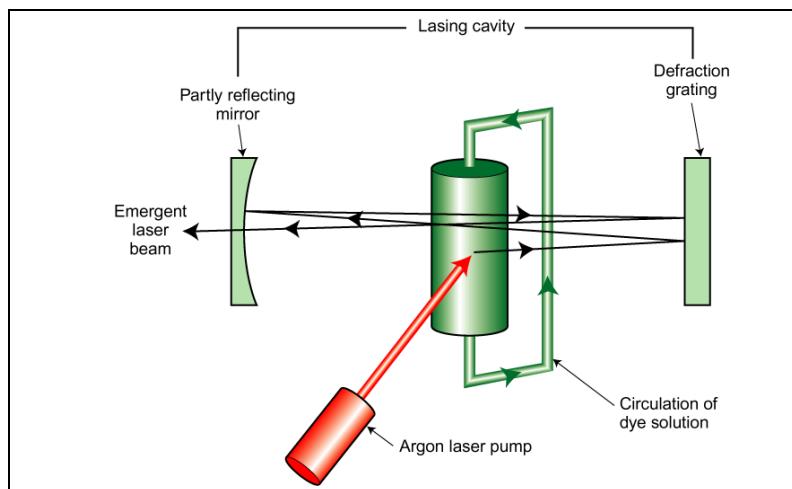


Figure 15 An argon laser is used to pump dye molecules into electronically excited states, which fluoresce. The emitted light travels to a diffraction grating, where the desired wavelength is selected. These selected photons pass back into the laser cavity, causing a cascade of stimulated emission, which is mode locked at the selected wavelength. Because the diffraction grating can be turned to reflect other selected wavelengths, the dye laser can be used to tune the emergent laser beam to whatever wavelength is required (the range is set by the fluorescence emission spectrum of the dye).

for lasing. The ruby laser is a three-level system. When spontaneous emission occurs, some of these emitted photons stimulate the production of a cascade of photons, appearing as red laser light.

The ruby rod is cut to a precise length, determined by the wavelength of the laser light (see “Mode locking” on page 707). There are mirrors at each end of the rod, one of which is only partially reflective so as to allow the laser beam to emerge. As lasing begins within the rod, light reflects up and down the rod and the intensity of the resulting cascade builds up. Some of the light escapes during the build up of the cascade of photons (amplification) but the build up to a pulse is so rapid that, within a few milliseconds, the main part of the pulse emerges as the laser beam. Figure 9 illustrates the principle. The flash lamp continues to pump up the excited state ready for the next pulse. Thus, the emergent beam of laser light consists of a series of short pulses of intense visible radiation, the time between pulses being controlled partly by the rate at which the pump light flashes.

If the flash lamp is pulsed very rapidly, the emergent beam appears at a rate governed by the lifetime of the inverted population. The resulting laser beam becomes almost continuous because the pulses follow each other so rapidly. However, such a solid state laser should not be pulsed too rapidly because, if it is, the rod heats up to an unacceptable extent, causing distortion and even fracture. Generally, solid state lasers are not used in continuous mode because of this heating aspect. Liquid or gas lasers do not suffer from this problem.

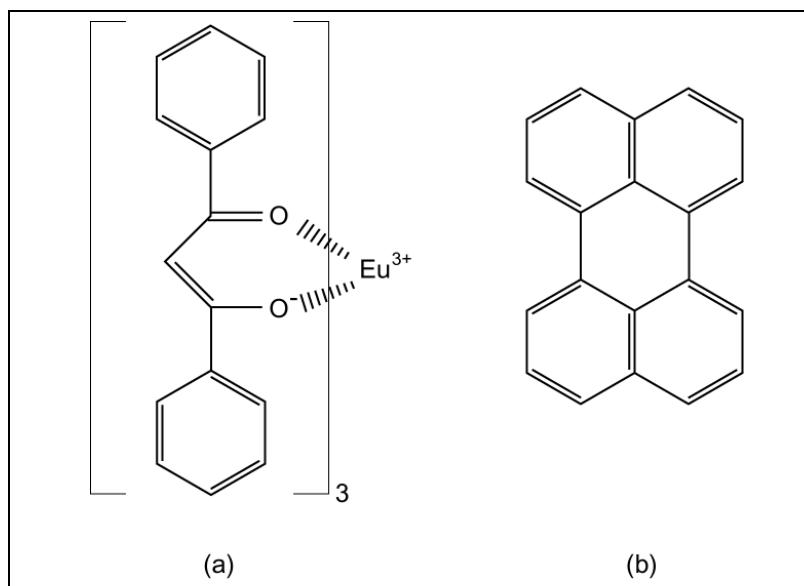


Figure 16 Two typical “dye” molecules. The europium complex (a) transfers absorbed light to excited state levels of the complexed Eu^{3+} , from which lasing occurs. The perylene molecule (b) converts incident radiation into a triplet state, which decays slowly and so allows lasing to occur.

Neodymium and YAG lasers.

The principle of these is very similar to that of the ruby laser. Neodymium ions (Nd^{3+}) are used in place of Cr^{3+} and are often distributed in glass rather than in alumina. The light from the neodymium laser has a wavelength of 1060 nm (1.06 μm), viz., it emits in the infrared region of the electromagnetic spectrum. Yttrium (Y) ions in alumina (A) compose a form of the naturally occurring "garnet" (G) and hence the name, YAG laser. Like the ruby laser, the Nd and YAG lasers operate from three and four level excited state processes.

Lasers in mass spectrometry

Until relatively recent times, lasers were not much used in mass spectrometry. It has been known for many years that light can cause ionisation in substances if the light energy (wavelength) was sufficient. Generally, this wavelength is in the far ultraviolet end of the UV/visible spectrum, in which region all substances absorb the radiation. The mass analysers in mass spectrometers operate under vacuum so that any "light" sources to be used for ionisation must also operate under similar vacuum conditions if the irradiation is to reach and ionise the sample molecules to be analysed. For this reason, ion sources based on ionisation of sample molecules by light were mostly research curiosities. When lasers first became commercially available, the useful laser sources mostly emitted visible light of an energy, which is insufficient for ionisation. As the laser beams became more intense (more photons per unit area per unit time), multiphoton absorption events could be achieved easily. Whereas one photon can be absorbed by a molecule but with insufficient energy for ionisation, two photons absorbed within a short space of time can cause ionisation (Figure 17). At this stage, lasers began to be used to excite molecules and even ions in flight in the mass spectrometer. This last absorption of light by molecular ions is enough to cause them to fragment. Multiphoton absorption and the ease of shining laser light through suitable windows into a mass spectrometer led to the first commercial ionisation sources. These were generally not too efficient in the numbers of ions formed per unit mass of substance irradiated but the process has been developed considerably and is particularly well known in MALDI (see below) and in the formation of aerosols from solids in plasma torch isotope analysis.

For irradiation of solids, the intensity of a laser beam means that a great deal of energy is absorbed by the substance being irradiated in a very short space of time. Multiphoton absorption in a few pico or nanoseconds leads to many molecules in the sample being elevated to highly excited states. As the latter begin to equilibrate, much of this added energy is channelled into rotational and vibrational modes so

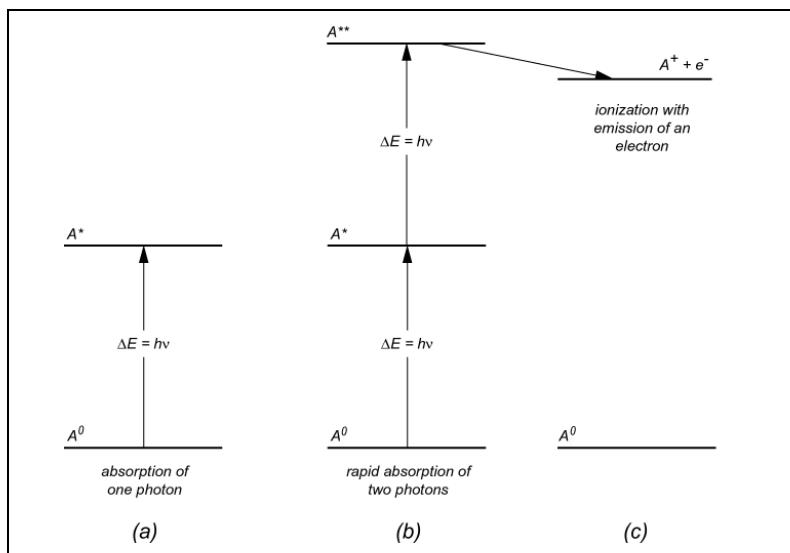


Figure 17 (a) Absorption of one photon raises an atom or molecule from the ground state (A) to an excited state (A^*), with energy gain, ΔE .
 (b) Rapid successive absorption of two photons raises the atom or molecule to a doubly excited state level (A^{**}), with energy $2\Delta E$. This state is energetically above the normal ionization energy (A^+) for A . Therefore, an electron is ejected as the cation forms; the energy of the emitted electron is the difference in energy between A^{**} and A^+ .

that the sample molecules behave as if they had been subjected to very high temperatures for a brief interval of time. There is rapid expansion at the irradiation site and volatilization occurs (ablation). This expansion is so rapid that a shock wave travels through the sample and underlying layers of the sample substance are subjected temporarily to very high temperatures and pressures. Usually, before the sample molecules can relax back to their ground states, a second laser pulse arrives and the process is repeated. This pulsing is so rapid that the vaporizing sample is subjected to the next laser pulse before all of the vapour (a plasma of neutrals, ions and electrons) has dissipated and the vapour molecules are further excited by absorbing more photons. Many of the excited states reached by multiple photon absorption lead to ejection of an electron (ionization) so that ablation produces some ions, which may be extracted by suitable electric fields and mass analysed.

Another consequence of the rapidity with which photons are absorbed lies in the amount of fragmentation observed. The rapid dissipation of energy into vibrational modes might be expected to lead to bond cleavage and fragmentation of the sample molecules. Some of this does occur but, before there is time for much bond-breaking to occur, there is conversion of the vibrational energy into kinetic energy as the sample molecules vibrate against each other. The molecules vaporize due to the kinetic energy and much of the excess of vibrational energy is dissipated this way. In these circumstances, fragmentation is limited. Ionization itself is still not highly likely and relatively few ions are formed. Many of the ions are protonated molecules rather than being singly ionized species. A big advance was made when a matrix was used to improve the numbers of ions formed on laser irradiation. This techniques became known as MALDI (Matrix Assisted Laser Desorption Ionization; see below). Ablation is important when used with a secondary ionization mode. Intractible solids such as ceramics can be ablated and the resulting vapour passed into, say, a plasma torch for complete ionization (see Back-to-Basics guide on *Plasma Torches*). This mode is extremely useful in the examination of atomic isotope patterns and ratios in samples that are otherwise difficult to analyse. Direct laser desorption ionization is used to examine the composition of surfaces and for depth profiling through specimens (see Back-to-Basics guide on *Laser Desorption Ionization*).

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For MALDI, the matrix is a substance of relatively low molecular mass, which is mixed intimately with the sample to be analysed. The matrix is preferably a good source of protons and is usually acidic but other Lewis acids (X^+), as with Ag^+ , may be used to promote formation of $[M + X]^+$ ions. When the matrix/sample mix is irradiated by laser light of a suitable wavelength, the matrix absorbs most of the energy and ablates or vaporizes. Sample molecules accompany this expanding “cloud” of matrix and, if the basicities are right, transfer of protons between matrix and sample occurs, leading to the formation of $[M + H]^+$ ions. Thus, MALDI mass spectra are characterised by protonated molecular ions and few fragment ions. The technique is so useful with even thermally sensitive molecules that it has come into widespread use for proteins and similar polar molecules. It might be noted that non-linear molecules have $3N-6$ vibration modes, in which N is the number of atoms. A molecule with 200 atoms has 594 vibration modes. If each of these is raised to the next excited vibrational level, each vibration requires little energy but a total of 594 vibrators contain enough total energy for bond breaking. For such bond breaking to occur, all or most of the excess of vibrational energy has to arrive in one bond at the same instance of time (about 10^{-13} seconds). The chances of this occurring in a multiatomic molecule are very low and, together with radiative loss of excess of vibrational energy, this effect is important in preserving the structural integrity of large, normally thermo-sensitive molecules. This phenomenon applies to “isolated” molecules and ions, as in ablated plasmas and vapours. For assemblies of molecules, as in solids or melts or dense gases, there is continual interaction between the molecules (collision) with energy transfer and there are constraints on vibration and rotation because of the close packing. This, and the longer time scales before vaporization can occur (a few milliseconds to infinity), means there is ample time for excess of vibrational energy to accumulate into one or more bonds so as to cause bond cleavage. This is what would happen if a protein sample were simply heated slowly in a “pot” to try to effect volatilization before mass spectral analysis.

The advent of lasers and MALDI into mass spectrometry has had a major effect, especially in the analysis of large, polar biochemicals. Whereas electron ionization gives many fragment ions, which carry structural information, direct laser ionisation and MALDI give mostly protonated molecular ions so that MS/MS with collision induced dissociation becomes necessary for inducing fragmentation in order to get the same structural information. Of course, electron ionisation methods are not useful for vaporizing and ionizing large biomolecules

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if these need to be first vaporized thermally since this leads to their decomposition and therefore loss of molecular mass and structural data.

- Conclusion** Modern commercial lasers may be obtained, which are able to provide intense beams of monochromatic, coherent radiation. The whole of the UV/visible/IR spectral range is accessible by suitable choice of laser. In mass spectrometry, this light can be used to cause ablation, direct ionisation and indirect ionisation (MALDI). Ablation (often together with a secondary ionization mode) and MALDI are particularly important for the examination of complex, intractable solids and large polar biomolecules respectively.

Back to Basics Section H: Miscellaneous

CHAPTER H5

IRMS - ISOTOPES & MASS SPECTROMETRY

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Quick Guide

- At the sorts of temperatures that exist normally on earth, all matter is made up from about 90 elements.
- Some of these elements are familiar, such as solid iron, liquid mercury and gaseous helium.
- Most elements combine in such ways as to create millions of other types of substances, such as chalk (a combination of the three elements calcium, carbon and oxygen) and water (a combination of hydrogen and oxygen).
- If the elements exist as such, they are still called elements but, if they have combined with each other, the different combinations are called compounds. Thus, mercury is an element but water is a compound.
- Whether existing separately as elements or combined as compounds, elements are composed of atoms, which are the smallest part of an element that can exist naturally.
- Atoms of mercury cling together to form the familiar liquid, atoms of iron hold together to form the solid metal and atoms of hydrogen and oxygen combine to form molecules, which hold together as water.
- Thus, all matter is composed of atoms, sometimes all of one sort as with iron and sometimes of more than one sort combined together, as with rust, which is a combination of atoms of the element iron and atoms of the element oxygen.
- Each atom is made up from a small dense nucleus surrounded by electrons. Most of the mass of each atom resides in the nucleus and, for most purposes, the mass of the electrons in an atom can be ignored.
- A nucleus is composed of protons and neutrons, each of which has unit atomic mass. The number of protons characterises each element. In going from one element to the next, the total number of protons increases by one. Thus, the simplest element, hydrogen, has atoms having only one proton in the nucleus and the next simplest, helium, has two protons in the nucleus.
- Whereas the number of protons in an atomic nucleus characterises each element, the mass of the nucleus is made up from the total number of protons and neutrons.

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- An atom of one of the simplest elements, helium has a mass of four atomic units (Daltons) and is made up of 2 protons and 2 neutrons, whereas each atom of the heavier element, phosphorus, has 15 protons and 16 neutrons, giving it a mass of 31 Daltons.
- The ratio of the number of protons to neutrons is not fixed. Thus, in some elements, as with phosphorus, the ratio is 15 to 16 (1:1.07) but, in the precious metal rhodium, the ratio is 45 to 58 (1:1.29).
- Phosphorus and rhodium are unusual amongst the elements in being made up of atoms, which naturally contain only one ratio of protons to neutrons and therefore have only one mass, which is 31 (15 protons plus 16 neutrons) for phosphorus and 103 (45 protons and 58 neutrons) for rhodium. Such elements are called mono-isotopic - each of their atoms has the one and only one mass in each case.
- Other elements have atoms, which may have different ratios of protons to neutrons. Thus, hydrogen actually is made up from three types of atoms. All atoms of hydrogen have always the same number of protons (one for hydrogen), giving each a mass of 1 Dalton but some atoms of hydrogen also contain 1 neutron in the nucleus as well as the proton (mass of 2) and yet others have two neutrons with each proton (mass of 3). Thus, we say hydrogen has three naturally occurring isotopes.
- Chemically, there is little difference between the reactivity of the different isotopes for any one element. Thus, isotopes of palladium all react in the same way, but react differently from all isotopes of platinum.
- One instrument that can reveal the presence of isotopes is a mass spectrometer, which may be regarded as a very accurate weighing machine!
- Whereas isotopes of hydrogen are almost indistinguishable for most chemical purposes, a mass spectrometer 'sees' them as three different entities of mass 1, 2 and 3. In a similar fashion, isotopes of other elements can be distinguished and therefore, a mass spectrometer is important for its ability to separate the isotopes of elements.

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- Some, but by no means all, isotopes are radioactive. Of the three isotopes of hydrogen, only that of mass 3 (tritium) is radioactive, the other two being non-radioactive. The radioactive isotopes can be examined by other instrumental means than mass spectrometry but these other means cannot 'see' the non-radioactive isotopes and are not so versatile as a mass spectrometer.
- Many artificial isotopes can be created through nuclear reactions. Such radioactive isotopes, as for example with artificial isotopes of iodine, are used in medicine and isotopes of plutonium are used in making atomic bombs!
- In many analytical applications, the ratio of occurrence of the isotopes is important. For example, it may be important to know the exact ratio of the abundances (relative amounts) of the isotopes 1, 2 and 3 in hydrogen. This knowledge requires a mass spectrometric measurement of the isotope abundance ratio.
- Whereas all mass spectrometers can measure abundance ratios to some degree of accuracy, special mass spectrometers have been designed to measure isotope ratios very accurately. These are used in such diverse areas as dating the antiquity of objects to unravelling details of reaction mechanisms to testing athletes for illegal use of body-building drugs.

Summary Atoms of elements are composed of isotopes. The ratio of natural abundance of the isotopes is characteristic of an element and is important in analysis. A mass spectrometer is normally the best general instrument for measuring isotope ratios.

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ISOTOPES AND MASS SPECTROMETRY

Introduction

The concept that all substances are made up from 'elements' and atoms goes back at least 2000 years. Originally, only four 'elements' were recognised, viz., air, earth, fire and water. Each substance was thought to be made up of very small particles called 'atoms', which could not be subdivided any further. This early mental concept of the nature of matter was extremely prescient, considering there were no experimental results to indicate that this should be so and none to verify that it was so. Modern atomic theory is much more rigorously based and we even have the ability to 'see' atoms with special tunnelling microscopes. All of chemistry is based on how atoms react with each other.

The idea that air, earth, fire and water are elements and that there are only four elements has long gone but the basic idea of atoms being the simplest building blocks of matter is still accepted, but with a proviso. In chemistry, reactions occur between atoms and, in that sense, atoms can be regarded as the simplest building blocks. However, the inner structures of atoms have important consequences and, under special conditions, atoms are not regarded as the simplest building blocks of matter that can exist alone. At this next level, atoms themselves are seen to be composed of three entities, viz., electrons, protons and neutrons. In particular, the numbers and masses of protons and neutrons determine the character of each element. The ratios of protons and neutrons in an atomic nucleus are important and gives rise to the existence of isotopes. Mass spectrometers are particularly effective general instruments for exploring the existence and abundance ratios of isotopes. The next sections give first a brief explanation of the structure of atoms and then show how isotopes arise.

Atomic Structure and the Elements

Many elements are familiar to us in everyday life. The solid, iron, is an element, used for making ships, cars, spades and so on. There are about 90 such elements, other familiar ones being helium, oxygen, nitrogen, mercury, platinum and gold. As an element, iron is composed of atoms of iron, the smallest building blocks, each of which is indivisible by chemical means. A lump of iron is made up from million, trillions and zillions of atoms and, as you might guess, the mass of each atom of iron is very small, actually about 10^{-22} grams!! In a piece of iron weighing about 50 grams there are about 10^{23} atoms.

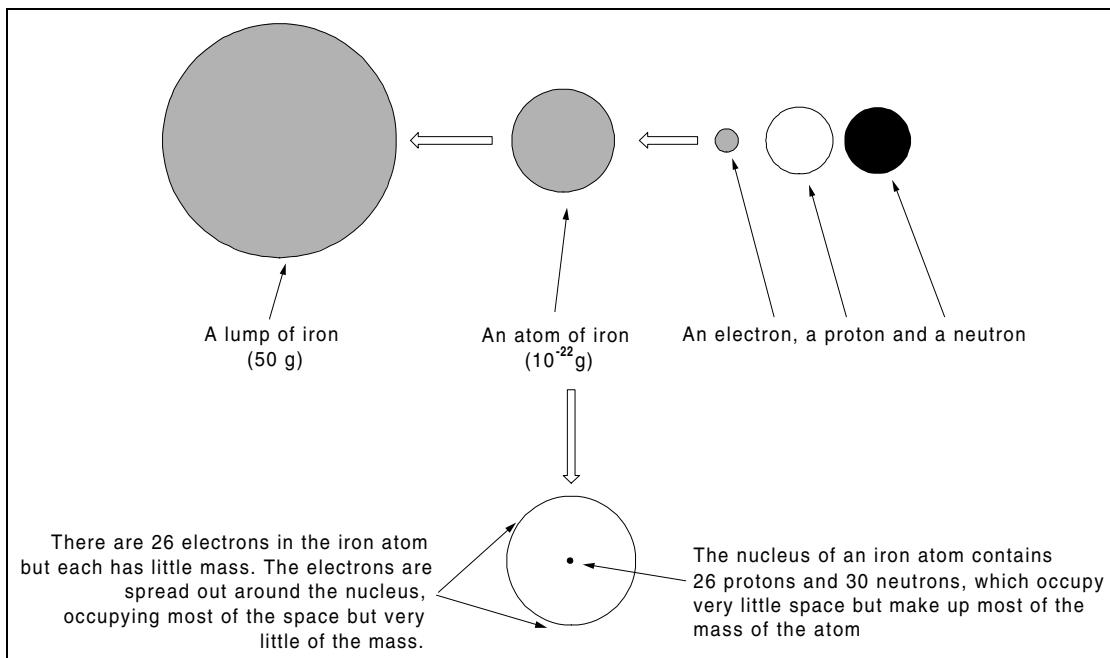


Figure I A representation of atomic structure. The various spheres are not drawn to scale. The lump of iron on the left would contain almost a million million million atoms, one of which is represented by the sphere in the top centre of the page. In turn, each atom is composed of a number of electrons, protons and neutrons. For example, an atom of the element iron contains 26 electrons, 26 protons and 30 neutrons. The physical size of the atom is determined mainly by the number of electrons but almost all of its mass is determined mainly by the number of protons and neutrons in its dense core or nucleus (lower part of figure). The electrons are spread out around the nucleus and their number determines atomic size but the protons and neutrons compose a very dense, small core and their number determines atomic mass.

If there were only the 90 some elements in isolation, there could only be about 90 different substances possible but everyday experience shows that there are millions of different substances, such as water, brick, wood, plastics and so on. In fact, elements can combine with each other and it is the complexity of these possible combinations that gives rise to the myriad substances found naturally or produced artificially. These combinations between atoms of the elements are called compounds. Since atoms of an element can combine with themselves or with those of other elements to form molecules there is a wide diversity of possible combinations to make all of the known substances, naturally or synthetically. Therefore, atoms are the simplest *chemical* building blocks. However, to understand atoms, it is necessary to examine the structure of a typical atom or, in other words, to examine the building blocks of the atoms themselves.

The building blocks of atoms are called *electrons*, *protons* and *neutrons* (Figure 1). It might be noted here in passing that, in high energy physics, even these 'simplest' building blocks are made up from even smaller ones such as quarks and gluons. However, chemically, these much smaller building blocks are not concerned in chemical reactions or chemistry and will not be considered further here. For mass spectrometry, only the structure of the atomic building blocks (electrons, protons and neutrons) is of importance.

Electrons

An electron carries one unit of negative electrical charge (Figure 2). Its mass is about 1/2000 th that of a proton or neutron. Therefore, very little of the mass of an atom is made up from the masses of the electrons it contains and generally, the total mass of the electrons is ignored. For example, an atom of iron has a mass of 56 atomic units (au; these are also called Daltons), of which only about 0.02% is due to the 26 electrons. Thus, an iron atom (Fe^0) is considered to have the same mass as does a doubly-charged cation of iron (Fe^{++}), even though there is a small mass difference.

Although each electron is very small, all of the electrons in an atom move around the nucleus, sometimes being close to the nucleus and, at other times being quite far away. On average, the distance of the electrons from the nucleus is many times the diameter of the nucleus. Therefore, although the electrons add very little to the mass of the atom, they do determine how big the atom is overall (Figure 1). Imagine an atom as big as a football, with a small pellet of lead at its centre. The air (electrons) surrounding the lead pellet (nucleus) represents the volume occupied by the electrons but the small pellet constitutes most of the mass.

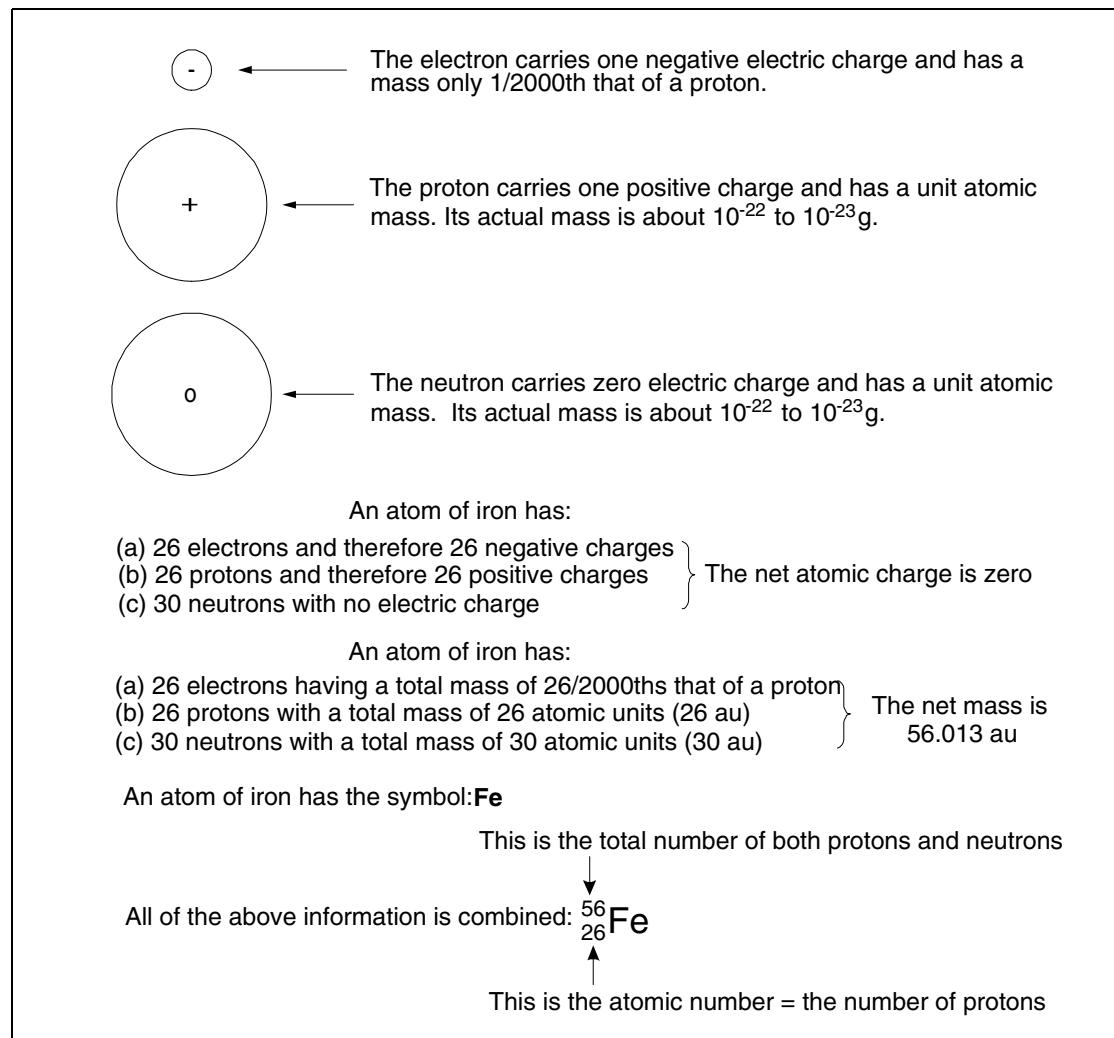


Figure 2 The top part of the figure gives an indication of the electric charges and relative sizes of the three major building blocks of atoms (electron, protons and neutrons) of importance for mass spectrometric purposes. The relative sizes as drawn are not meant to be to scale. Below this top part is an indication of how the electrons in an atom of iron are mostly responsible for its size and of how the nucleus is responsible for most of the mass. In this simplified discussion, there is no consideration of 'packing fractions', which are partly responsible for atomic masses not being whole numbers. The bottom part of the figure illustrates by the example of iron how an atom of any element is designated so as to show the numbers of protons, neutrons and electrons it contains.

From a chemical and mass spectrometric viewpoint, the other major property of an electron is its electric charge of one unit. Removal of one electron from an atom or molecule $[M]$ gives a singly charged cation $[M^+]$ and addition of an electron gives a singly charged anion $[M^-]$. The electric charge is necessary for the mass spectrometer to be able to measure a mass-to-charge (m/z) ratio. Because m/z gives the mass of M^+ or M^- , and the difference between these masses is very small compared with the mass of the neutral species M , it can be said that the m/z value gives the mass M . Although not strictly true, for most purposes this is a close enough approximation (Figure 3).

Protons Each proton is about 2000 times heavier than an electron and its mass is one atomic unit. Importantly, it also carries one unit of positive electric charge (Figure 2). The proton is very small and is confined to the nucleus of the atom (along with any neutrons) and the mass of the atom resides almost entirely in this very dense nucleus. The nucleus is not the most important factor in determining atomic size (the electrons do that) but it does determine where most of the mass of the atom resides.

The unit positive charge on the proton balances the unit negative charge on the electron. In neutral atoms, the number of electrons is exactly equal to the number of protons. In an iron atom (Fe^0), there are 26 electrons and just 26 protons. A cation is formed by removing electrons not by adding protons. An ion M^+ has one electron less than the neutral atom M^0 . Similarly, an anion M^- is formed by adding an electron and not by subtracting a proton from M^0 .

The *number of protons* in an atom determines which atomic species is present. The simplest element hydrogen has atoms, each of which has just one proton. No element other than hydrogen has *only* one proton. The next element (helium) has two protons in each atom and so on through all of the known elements. Iron, as has been shown, has 26 protons and this is the reason it is iron and not, say, lead. In fact, each element is characterised by its atomic number, which is the number of protons in an atom of the element (Figure 2). Hence, an atom of iron is represented by the symbol for iron (Fe) with a number written as a subscript in front of it $_{26}Fe$; this signifies iron of atomic number 26. The designation $_{17}Cl$ signifies chlorine of atomic number 17 (17 protons in the nucleus).

The mass of an atom or ion resides mostly in the nucleus

Atom of iron: Fe^0 , mass approximately 56.013 from 26 protons, 30 neutrons and 26 electrons

A cation of iron: Fe^{++} , mass approximately 56.012 from 26 protons, 30 neutrons and 24 electrons

The m/z value for Fe^{++} is $56.012/2 = 28.006$

Some naturally occurring multi-isotopic elements (approximate % natural abundances are shown in brackets). Note that only one carbon isotope is radioactive

Carbon	$^{12}_6\text{C}$ (98.9)	$^{13}_6\text{C}$ (1.1)	$^{14}_6\text{C}$ (very small)*
Oxygen	$^{16}_8\text{O}$ (99.8)	$^{17}_8\text{O}$ (0.04)	$^{18}_8\text{O}$ (0.2)
Sulphur	$^{32}_{16}\text{S}$ (95.0)	$^{33}_{16}\text{S}$ (0.8)	$^{34}_{16}\text{S}$ (4.2)
Bromine	$^{79}_{35}\text{Br}$ (50.5)	$^{79}_{35}\text{Br}$ (49.5)	
Molybdenum	$^{92}_{42}\text{Mo}$ (15.8)	$^{94}_{42}\text{Mo}$ (9.0)	$^{95}_{42}\text{Mo}$ (15.7) $^{96}_{42}\text{Mo}$ (16.5) $^{97}_{42}\text{Mo}$ (9.5) $^{98}_{42}\text{Mo}$ (23.8) $^{100}_{42}\text{Mo}$ (9.6)
Mercury	$^{196}_{80}\text{Hg}$ (0.2)	$^{198}_{80}\text{Hg}$ (10.0)	$^{199}_{80}\text{Hg}$ (16.8) $^{200}_{80}\text{Hg}$ (23.1) $^{201}_{80}\text{Hg}$ (13.2) $^{202}_{80}\text{Hg}$ (29.8) $^{204}_{80}\text{Hg}$ (6.9)

Figure 3 The upper part of the figure illustrates why the small difference in mass between an ion and its neutral molecule is ignored for mass spectrometric purposes. In mass measurement, ^{12}C has been assigned arbitrarily to have a mass of 12.00000. All other atomic masses are referred to this standard. Below that, there is a small selection of elements with their naturally occurring isotopes and their natural abundances. Although not shown above, at one extreme, xenon has nine naturally occurring isotopes, whereas at the other, some elements such as fluorine have only one.

Neutrons A neutron is characterised by having no electrical charge but has one unit of atomic mass, the same as that of a proton (Figure 2). Neutrons, like protons, reside in the atomic nucleus and contribute to the mass of the atom. The chemistry of an atom, like its size, is determined by the electrons in the atom. The mass of the atom is characterised mainly by the total number of neutrons and protons in the nucleus (atomic binding energies are ignored in this discussion). For mass spectrometric purposes of measurement, it is the mass that is important in giving m/z values.

The Atomic Nucleus and Isotopes

Consider a nucleus of the simplest element, hydrogen, of atomic number 1; it is designated ${}_1^1\text{H}$. There is one proton (and of course one electron) and the atomic mass is 1 Dalton (mostly from the one proton). The total number of protons plus neutrons is then indicated by another (superscripted) prefix. In this present case it is ${}_1^1\text{H}$ (Figure 2). The chemistry of hydrogen is mostly determined by that one electron but its mass is determined mostly by the one proton. There is another type of hydrogen atom called deuterium, which includes a neutron in its nucleus and has its own symbol, D. However, it is less confusing to write it as ${}_1^2\text{H}$. This type of hydrogen atom also has the one electron and one proton and its chemistry is the same as that of ${}_1^1\text{H}$. Because the nucleus of ${}_1^2\text{H}$ contains one neutron and one proton, its atomic mass is 2. It can be seen that there are two types of hydrogen atom, one being about twice as heavy as the other but the general chemistry of the two is identical for most purposes. These two kinds of hydrogen atom are called *isotopes*. The predominantly abundant isotope is ${}_1^1\text{H}$ and the isotope in much lesser abundance is ${}_1^2\text{H}$. These isotopes are not radioactive. However, there is even a third isotope of hydrogen called tritium (${}_1^3\text{H}$), which has two neutrons and one proton in its nucleus and is some three times heavier than the first hydrogen isotope, ${}_1^1\text{H}$. All these hydrogen isotopes react chemically in the same way and only the ${}_1^3\text{H}$ isotope is radioactive.

Some elements in their natural state have only one isotope, as with fluorine, phosphorus and rhodium but others have several isotopes, as with carbon (three), oxygen (three) and molybdenum (seven). A few examples are given in Figure 3. A small proportion of naturally occurring isotopes is radioactive but most isotopes are not. The radioactivity results from the nucleus being unstable, which happens if the numbers of protons and neutrons in a nucleus become seriously unbalanced. Nuclei of carbon having six protons and six neutrons (${}^{12}\text{C}$) or six protons and seven neutrons (${}^{13}\text{C}$) are stable and not radioactive but the carbon isotope with six protons and eight neutrons (${}^{14}\text{C}$) is unstable and is radioactive (Figure 3).

The two isotopes of chlorine are $^{35}_{17}$ Cl and $^{37}_{17}$ Cl, which occur naturally in the abundance ratio of 3

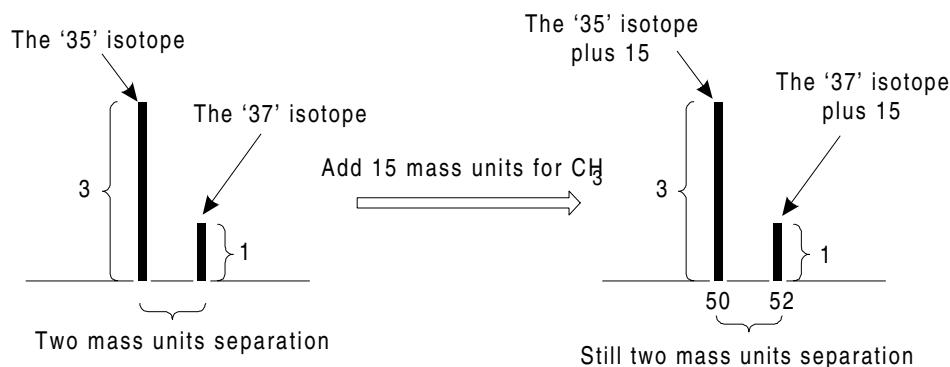


Figure 4 A diagrammatic illustration of the effect of an isotope pattern on a mass spectrum. The two naturally occurring isotopes of chlorine combine with methyl to give methyl chloride. Statistically, because their abundance ratio is 3:1, three ^{35}Cl isotope atoms combine for each ^{37}Cl atom. Thus, the ratio of the molecular ion peaks at m/z 50, 52 found for methyl chloride in its mass spectrum will also be in the ratio of 3:1. If nothing had been known about the structure of this compound, the appearance in its mass spectrum of two peaks at m/z 50, 52 (two mass units apart) in a ratio of 3:1 would immediately identify the compound as containing chlorine.

In more recent times, it has become possible to create isotopes that do not exist naturally. These are the *artificial isotopes* and all are radioactive. For example, some 13 artificially created isotopes of iodine are known, as well as its naturally occurring mono-isotopic form of mass 127. Mass spectrometry is able to measure m/z values for both natural and artificial isotopes.

Isotope Ratios

Approximate Abundance Ratios

Naturally occurring isotopes of any element are present in unequal amounts. For example, chlorine exists in two isotopic forms, one with 17 protons and 18 neutrons (^{35}Cl) and the other with 17 protons and 20 neutrons (^{37}Cl). The isotopes are not radioactive and they occur respectively in a ratio of nearly 3:1. In a mass spectrum, any compound containing one chlorine atom will have two different molecular masses (m/z values). For example, methyl chloride (CH_3Cl) has masses of 15 (for the CH_3) plus 35 (total = 50) for one isotope of chlorine and 15 plus 37 (total = 52) for the other isotope. Since the isotopes occur in the ratio of 3:1 then molecular ions of methyl chloride will show two molecular mass peaks at m/z values of 50 and 52, the heights of the peaks being in the ratio of 3:1 (Figure 4). This example can be used in reverse to show the usefulness of looking for such isotopes. Suppose there was an unknown sample that had two molecular ion peaks in the ratio of 3:1 which were two mass units apart, then it could be reasonably deduced that it was highly likely the unknown contained chlorine. In this case the isotope ratio has been used to identify a chlorine-containing compound. This use of mass spectrometry is widespread in general analysis of materials but it makes use of only approximate ratios of isotopes because that is all that is necessary for identification. It is not usually necessary to know if the ratio is, say 3.001:1.00 or 3.002:1.00; all that is needed is a ratio near to 3:1. Where there are several isotopes of an element, the actual *pattern* of masses and abundances is enough for identification. It would be very difficult to miss the evidence for mercury or molybdenum in a mass spectrum when there are seven isotopes, having distinctive patterns of abundances and mass differences (Figure 3). These uses of isotopes are discussed in the Back-to-Basics Guide, *Use of Isotope Patterns in Mass Spectrometry*. However, there are other uses of isotopes that require very accurate determinations of their ratios of abundances.

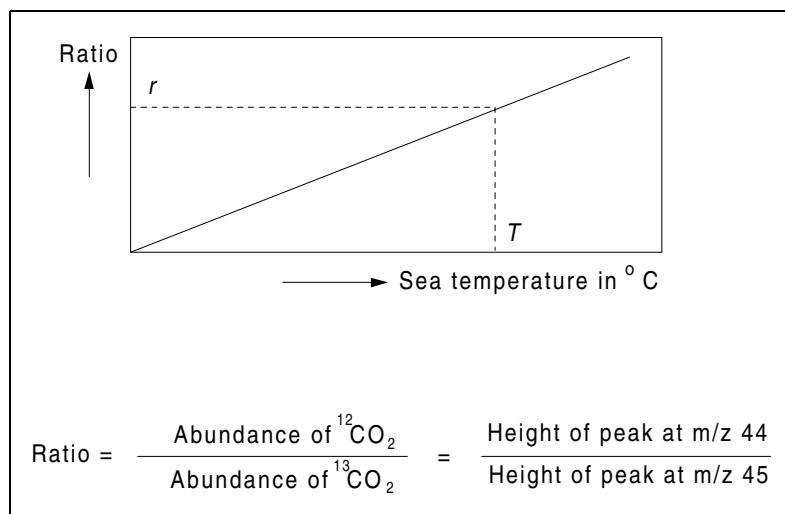


Figure 5 By experimentally determining the ratio of abundances of ^{12}C and ^{14}C isotope peaks for CO_2 dissolved in sea water at various temperatures, a graph can be drawn relating the solubility of $^{12}\text{CO}_2$ compared with that of $^{13}\text{CO}_2$ (the ratio described above). On extracting the CO_2 from sediment containing the shells (calcium carbonate) of dead sea creatures by addition of acid, a ratio (r) of abundances of $^{12}\text{CO}_2$ to $^{13}\text{CO}_2$ can be measured. If this value is read from the graph, a temperature T is found, which must have been the temperature of the sea at the time the sediment was laid down. Such experiments have shown that, 10,000 years ago, the temperature of the Mediterranean then was much as it is now.

Accurate Abundance Ratios

The use of accurate isotope ratio measurement is exemplified here by a method for the determination of the temperature of the Mediterranean Sea some 10,000 years ago. It is known that the relative solubility of the two isotopic forms of carbon dioxide ($^{12}\text{CO}_2$, $^{13}\text{CO}_2$) in sea water depends on temperature (Figure 5). One method for measuring the temperature of the sea is to measure this ratio. Of course, if you went to do it now, you would take a thermometer and not a mass spectrometer. But how do you determine the temperature of the sea as it was 10,000 years ago? The answer lies with tiny sea creatures called diatoms. These have shells made from calcium carbonate, itself derived from carbon dioxide in sea water. As the diatoms die, they fall to the sea floor and build up a sediment of calcium carbonate. If a sample is taken from a layer of sediment 10,000 years old, the carbon dioxide can be released by addition of acid. If this carbon dioxide is put into a suitable mass spectrometer, the ratio of carbon isotopes can be measured accurately. From this value and the graph of solubilities of isotopic forms of carbon dioxide with temperature (Figure 5), a temperature can be read off. This was the temperature of the sea during the time the diatoms were alive. To carry out such experiments in a significant manner, it is essential that the isotope abundance ratios be measured very accurately.

This accurate measurement of the ratio of abundances of isotopes is used for geological dating, estimation of the ages of antiquities, testing athletes for the use of banned steroids, examining fine details of chemical reaction pathways and so on. These uses are discussed in these Back-to-Basics Guides under various headings concerned with isotope ratio mass spectrometry.

Conclusion

Isotopes of an element are formed by the protons in its nucleus combining with various numbers of neutrons. Most natural isotopes are not radioactive and the approximate pattern of peaks they give in a mass spectrum may be used to identify the presence of many elements. The ratio of abundances of isotopes for any one element, when measured accurately, can be used for analytical purposes, such as dating geological samples or gaining insights into chemical reaction mechanisms.

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Quick Guide

- An element is characterised by its atomic number (Z), in which $Z = 1, 2, 3$, etc. The first element (hydrogen) has $Z = 1$, the next (helium) has $Z = 2$ and so on up to the heaviest natural element (uranium) with $Z = 92$.
- An atom contains a nucleus, surrounded by electrons. Most of the mass of the atom is centred in the nucleus.
- The nucleus consists of protons and neutrons; the number of protons (P) is equal to the atomic number ($P = Z$).
- The nucleus also contains neutrons. The number of neutrons (N) for any one element is similar to but not necessarily equal to the number of protons.
- Each proton or neutron has an atomic mass close to 1 unit. Neglecting the small electron mass and other factors, the total atomic mass of an element is given by the sum ($P + N$).
- For each element, the number of protons is fixed. Thus, for hydrogen ($Z = 1$) there is just 1 proton ($P = 1$); for the next element, helium ($Z = 2$), there are just 2 protons ($P = 2$) and so on up to the heaviest natural element, uranium, which has atomic number 92 and therefore has $Z = P = 92$.
- Since the total integer atomic mass (M) is given by the number of protons and neutrons then $M = P + N$. Because of the masses of the electrons in an atom and a "packing fraction" of mass in each nucleus, the actual atomic mass is not an integer.
- Some elements contain a fixed number of neutrons, as with fluorine ($P = 9, N = 10$) and phosphorus ($P = 15, N = 16$). For their natural occurrences, atoms of any one such element all have the same mass ($F = 19; P = 31$).
- Atoms of many other elements contain nuclei, which have different numbers of neutrons. For example, carbon ($Z = 6$) may have 6 neutrons ($M = 6 + 6 = 12$) or 7 neutrons ($M = 13$) or 8 neutrons ($M = 14$). Atoms of the same atomic number but having different numbers of neutrons (and different atomic masses) are called isotopes. Thus, naturally occurring carbon has three isotopes, for which $Z = P = 6$ and $N = 6$ or 7 or 8. These are written $^{12}_{6}\text{C}$, $^{13}_{6}\text{C}$, $^{14}_{6}\text{C}$.

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- For any one element, the abundances (relative amounts) of isotopes may be described in percentage terms. Thus, fluorine is monoisotopic, viz., it contains only nuclei of atomic mass 19 and phosphorus has 100% abundance of atoms with atomic mass 31. For carbon, the first two isotopes occur in the proportions of 98.882 to 1.108.
- These isotope masses and their ratios of abundances are characteristic of carbon. Similarly, the isotopes of other elements that occur naturally have "fixed" ratios of isotopes, as given in Table I and 2 at the end of the accompanying full text.
- In a mass spectrum, the ratios of isotopes give a "pattern" of isotopic peaks, which is characteristic of any one element. For example, the mass spectrum of any compound containing carbon, hydrogen, nitrogen and oxygen will show patterns of peaks due to the ^{12}C , ^{13}C , ^{14}N , ^{15}N , ^{16}O , ^{17}O and ^{18}O isotopes. The ratios of isotope abundances may be estimated directly from a routine mass spectrum and are frequently useful for identifying the presence of specific elements. Such uses are discussed in the main text.
- When measured carefully, isotope ratios are found not to be fixed but to vary slightly, depending on several factors. This variation is often very small and may be difficult to detect.
- Special isotope ratio mass spectrometers are needed to measure the small variations, which are too small to be read off from a spectrum obtained on a routine mass spectrometer. Ratios of isotopes measured very accurately (usually as 0/00, i.e., as per mil rather per cent) give information on, for example, reaction mechanisms, dating of historic samples or testing for drugs in metabolic systems. Such uses are illustrated in the main text.

Summary

Isotope ratios are very useful for identifying elements from their pattern of isotopes in a spectrum obtained on an ordinary mass spectrometer or for obtaining detailed information after accurate measurement of isotope ratios from special isotope ratio instruments.

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USES OF ISOTOPE RATIOS

Introduction

A general discussion of isotopes appears in the Back-to-Basics guide, *Isotopes*. For naturally occurring elements, the ratios of abundances of isotopes are well-known and, for convenience, are listed in Table 1 and 2. These ratios may be used in routine mass spectrometry for identifying various elements. For example, molybdenum has the seven isotopes listed here (the % abundances are given in brackets): ^{92}Mo (14.8), ^{94}Mo (9.25), ^{95}Mo (15.9), ^{96}Mo (16.7), ^{97}Mo (9.55), ^{98}Mo (24.1), ^{100}Mo (9.63); it would not be very difficult to identify the presence of molybdenum in a sample from the pattern of isotopic peaks in a mass spectrum. Although listed in Table 1 and 2 as "fixed" ratios, the actual ratios of isotopes differ slightly, depending on the source of the elements being investigated. Thus, although ^{12}C and ^{13}C are listed as occurring in a ratio of 98.882 to 1.108, if this ratio is measured in metabolic products, as in drug testing, the ratio will be found to be slightly different from that given in the Tables because metabolic reactions differentiate somewhat between ^{12}C and ^{13}C . Such accurate measurement of precise isotope ratios is important in many areas, including chemistry, geology, environmental science, the nuclear industry and medicine. Special instruments are necessary to measure these precise levels. It also becomes necessary to stipulate that certain materials should be regarded as having standard isotope ratios, which are used for gauging any changes appearing in "non-standard" substances.

For the naturally occurring elements, many new artificial isotopes have been made and are radioactive. These new isotopes can be measured in a mass spectrometer but may lead to unacceptable radioactive contamination of the instrument. This practical consideration needs to be carefully considered before using mass spectrometers for radioactive isotope analysis.

Apart from naturally occurring elements, there are now newly made elements beyond uranium. These constitute the trans-uranic series. All of the elements in this series are radioactive.

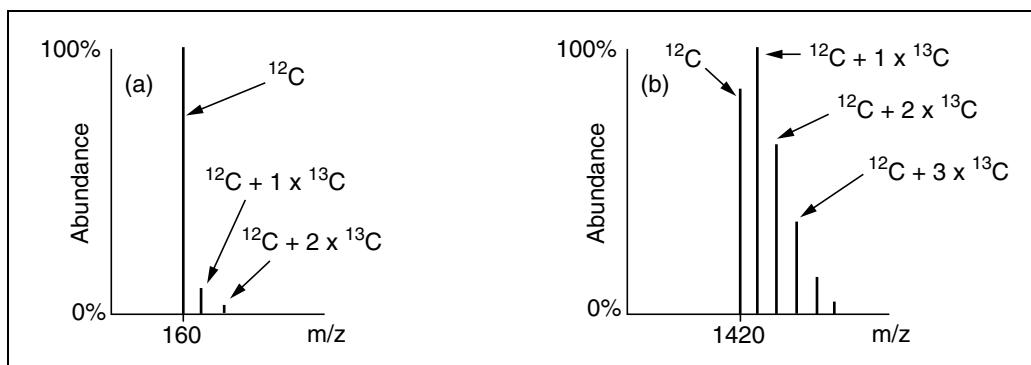


Figure 1. (a) A carbon compound having one fluorine and ten carbon atoms has a major molecular ion peak at m/z 160 in its mass spectrum, which corresponds to a preponderance of the ^{12}C isotope. The peaks at m/z 161 and 162, containing respectively one and two ^{13}C isotopes, are much smaller because the chances of finding one or two isotopic atoms among the total of only 10 atoms are low. In (b), a carbon compound having 100 carbon atoms in the molecule, the molecular ion peak at m/z 1420, representing only ^{12}C atoms, is smaller than the peak at m/z 1421, corresponding to those molecular ions having 99 ^{12}C atoms plus one ^{13}C atom; peaks representing ions having successively $2 \times ^{13}\text{C}$ (plus 98 ^{12}C), $3 \times ^{13}\text{C}$ (plus 97 ^{12}C) and $4 \times ^{13}\text{C}$ (plus 96 ^{12}C atoms) in the molecule are progressively less abundant but still prominent in comparison with a compound containing only ten carbon atoms in total.

Isotope ratios in routine mass spectrometry

General chemistry

The occurrence of the elements carbon, nitrogen and oxygen manifests itself in the isotope patterns occurring for all molecular or fragment ions. For small numbers of carbon atoms, the most abundant ions are those containing the ^{12}C isotope because it is nearly 100 times more abundant than the ^{13}C isotope. However, the relative abundance of the ^{13}C isotopic peak in a mass spectrum increases as the number of carbon atoms increases. Thus, for n carbon atoms, the abundance of the ions containing one ^{13}C isotope of carbon increases relative to those ions containing only the ^{12}C isotope by about $n \times 1.1\%$. The presence of isotopes from other elements can make the isotopic pattern quite complex. For the compound, fluorodecane, the fluorine is monoisotopic (^{19}F) and the natural abundance of the ^2H is very low compared with ^1H . Figure 1a shows the mass spectrum of the molecular ion region for fluorodecane, from which it is seen that the dominant peak is that corresponding to the ^{12}C isotope and those ions containing one ^{13}C atom constitute about 11% of this peak height at an m/z value one unit greater. Similarly, the peak at two mass units greater is due to those ions that have two ^{13}C atoms, the remainder being ^{12}C . The pattern of isotopic peaks is simply a distribution of the probabilities (chances) of finding different combinations of ^{12}C and ^{13}C atoms in the ten carbon atoms of fluorodecane. If there are ten carbon atoms in the molecule then each of the ten has a 1.1% chance of being ^{13}C and the chance that one ^{13}C will turn up is $10 \times 1.1\%$.

This probability distribution means that, for a carbon compound containing 100 carbon atoms as in fluoroheptane, the relative abundance of those ions containing 99 ^{12}C atoms and just one ^{13}C atom becomes $100 \times 1.1\% = 110\%$, as against 100% for those ions containing only ^{12}C atoms (Figure 1b). Thus, in the molecular ion region of its mass spectrum, the peak corresponding to the molecular ion based on ^{12}C is smaller than the next peak representing the relative number of those ions containing one ^{13}C atom. Ions containing two, three, four or more ^{13}C atoms also become relatively more abundant, as shown in Figure 1b.

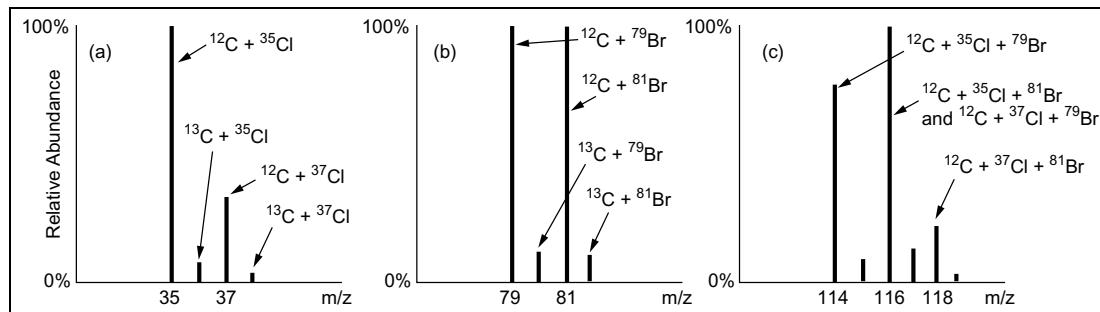


Figure 2. Partial mass spectra showing the isotope patterns in the molecular ion regions for ions containing carbon and, (a) only one chlorine atom, (b) only one bromine atom and, (c) one chlorine and one bromine atom. The isotope patterns are quite different from each other. Note how the halogen isotope ratios appear very clearly as 3:1 for chlorine in (a), 1:1 for bromine in (b) and 3:4:1 for chlorine and bromine in (c). If the numbers of halogens were not known, the pattern could be used in a reverse sense to decide their number.

(a)	Average atomic mass for Cl =	35.45
	Average atomic mass for C =	12.01
	Average atomic mass for H =	1.01
	∴ Average molecular mass for $\text{C}_2\text{H}_5\text{Cl}$	= 64.52 (used for weighing)
(b)	Isotopic mass for ^{12}C	= 12
	Isotopic mass for ^{13}C	= 13
	Isotopic mass for ^{35}Cl	= 35
	Isotopic mass for ^{37}Cl	= 37
	Isotopic mass for ^1H	= 1
	∴ Isotopic molecular masses for $\text{C}_2\text{H}_5\text{Cl}$	= 64, 65, 66, 67 (used in mass spectrometry)

Figure 3. An illustration of the use of average and isotopic mass. In (a), the average molecular mass for chloroethane is calculated and, in (b) the isotopic masses are calculated. An ordinary use of average mass in, for example, mass balances for chemical reactions, would use the average molecular mass of 64.52. However, a mass spectrometer can distinguish the different isotopic masses easily and so it is necessary to use these isotopic masses. For example, the molecular ion region in a mass spectrum contains isotopic molecular ions, as shown in Figure 1 and 2. Only approximate isotopic and average masses are used for purposes of illustration.

If there are oxygen or nitrogen atoms in the molecule their isotopes will add to this complexity but, in practice, these atoms present little complication because they are usually present in much smaller numbers than for carbon and the relative abundances of the minor isotopes are quite small. In mass spectrometry of organic compounds containing only small numbers of carbon atoms, the molecular ion peak is simply taken to be that corresponding to the ^{12}C isotope (the biggest peak or line in Figure 1a) and this is clearly differentiated from the other, much smaller isotopic peaks. However, as the number of carbon atoms increases, selection of the "all" ^{12}C isotope peak means that it does not constitute the biggest peak in the molecular ion region (Figure 1b). This effect can lead inadvertently to mis-identification of molecular mass for an unknown sample containing a large number of carbon atoms if the largest peak in the molecular ion region is assumed to be due only to ^{12}C atoms.

For other elements, which occur with major relative abundances of more than one isotope in the natural state, the isotope pattern becomes much more complex. For example, with chlorine and bromine, the presence of these elements is clearly apparent from the isotopes ^{35}Cl and ^{37}Cl for chlorine and ^{79}Br , ^{81}Br for bromine. Figure 2a shows the molecular ion region for the compound chlorodecane. Now, there are new situations in that ^{12}C , ^{13}C , ^{35}Cl and ^{37}Cl isotopes all have probabilities of occurring together. Thus, there are molecular ion peaks for $^{12}\text{C} + ^{35}\text{Cl}$, $^{13}\text{C} + ^{35}\text{Cl}$, $^{13}\text{C} + ^{37}\text{Cl}$ and so on. Even so, the isotopic ratio of 3:1 for ^{35}Cl to ^{37}Cl is very clear (Figure 2a). Similarly, the pattern for bromine, having a 1:1 ratio of the isotopes ^{79}Br and ^{81}Br is equally clear in bromodecane (Figure 2b). Indeed, the numbers of such atoms occurring in a molecule can be "counted" accurately from the pattern of isotopic peaks in the mass spectrum. Figure 2c shows the molecular ion region for a substance containing one chlorine and one bromine atom.

A common mistake for beginners in mass spectrometry is a confusion of average atomic mass and isotopic mass. For example, the average atomic mass for chlorine is close to 35.45 but this is an average of the numbers and masses of ^{35}Cl and ^{37}Cl isotopes. This average must be used for instruments, which cannot differentiate isotopes (for example, gravimetric balances). Mass spectrometers do differentiate isotopes by mass and so it is important in mass spectrometry that isotopic masses be used in calculating mass and not average atomic masses. A simple example of this difference is illustrated in Figure 3.

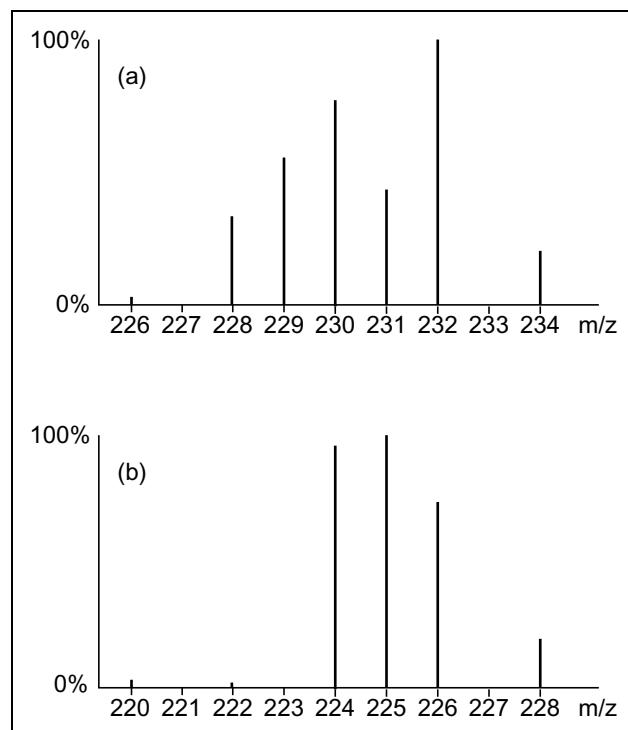


Figure 4. The isotope patterns for two simple organometallic compounds in the molecular ion region, (a) dimethyl mercury and (b) dimethylplatinum. The seven isotopes of mercury show up clearly and appear quite different from the six isotopes of platinum. Since there are only two carbon atoms, the contribution from ^{13}C is negligible.

For organometallic compounds, the situation becomes more complicated because the presence of elements such as platinum, iron, copper and so on introduces more complex isotopic patterns.

In a very general sense, for inorganic chemistry, as atomic number increases, the number of isotopes occurring naturally for any one element can increase considerably. An element of small atomic number like lithium has only two natural isotopes but tin has ten, xenon has nine and mercury has seven isotopes. This general phenomenon should be approached with caution because, for example, yttrium of atomic mass 89 is monoisotopic and iridium has just two natural isotopes at masses 191 and 193. Nevertheless, the occurrence and variation in patterns of multi-isotopic elements often make their mass spectrometric identification easy, as is depicted for the cases of dimethylmercury and dimethylplatinum (Figure 4).

Accurate determination
of isotope ratios

Special instruments (isotope ratio mass spectrometers) are used for the determination of isotope ratios, when needed, to better than about 3%. Such special instruments are described in these Back-to-Basics guides under the general heading of isotopes. The methods of ionization and analysis for such precise measurements are not described here.

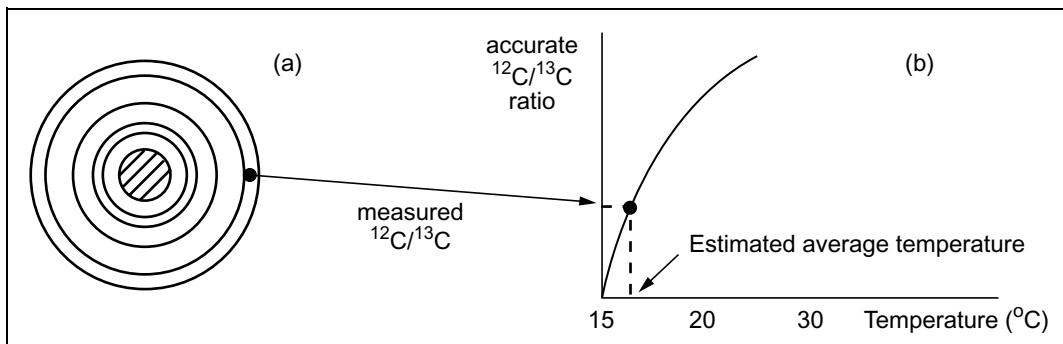


Figure 5 (a) A cross-section of a tree trunk reveals a number of "rings" for each year of growth. Comparison of the ring patterns for trees of different ages shows that the pattern is consistent for a given region. Thus, by looking at the patterns for trees or timbers from different ages, the patterns can be matched or overlapped to build up a profile of tree ring thickness with age. Radioactive dating allows approximate estimation of the date when a tree was growing but the tree ring method provides a more accurate assessment of dates for more recent times. (b) A graph showing the ratio $^{12}\text{C}/^{13}\text{C}$ for tree growth and average temperature. The graph can be used to give an accurate assessment of average "summer" temperatures in the geographical region of interest. From dendrological tree ring dating and accurate $^{12}\text{C}/^{13}\text{C}$ isotope ratios, the average summer temperatures for a region can be gauged quite accurately.

Some examples of the use of accurate isotope ratios

Archaeology

Dating of wooden objects has commonly been carried out by counting the number of tree rings, where this can be done (dendrochronology). As climate changes, the thicknesses of the rings change also as more wood is laid down in warmer, wetter weather than in colder, drier weather. By matching tree ring patterns, it has been possible to accurately date wooden objects for periods going back thousands of years. Although the rings are thicker when laid down in warmer weather and vice versa in cooler weather, it is not possible to gauge the actual average temperature, which each ring represents from the thickness. Isotopic analysis can do this. The $^{12}\text{C}/^{13}\text{C}$ ratio for tree rings has been measured for recent times, for which accurate summer and winter temperatures are known. From these data, a graph can be constructed that relates average temperature to the ratio of $^{12}\text{C}/^{13}\text{C}$. Such measurements can reveal differences of only 0.2 °C and can be used to form an accurate assessment of average annual temperatures for thousands of years (Figure 5).

Geology

In a similar vein, mean seawater temperatures can be estimated from the ratio of ^{16}O to ^{18}O in limestone. The latter rock is composed of calcium carbonate, laid down from shells of countless small sea creatures as they die and fall to the bottom of the ocean. The ratio of the oxygen isotopes locked up as carbon dioxide varies with the temperature of sea water. Any organisms building shells will fix the ratio in the calcium carbonate of their shells. As the limestone deposits form, the layers represent a chronological description of the mean sea temperature. To assess mean sea temperatures from thousands or millions of years ago, it is necessary only to measure accurately the $^{16}\text{O}/^{18}\text{O}$ ratio and use a pre-calibrated graph that relates temperatures to $^{16}\text{O}/^{18}\text{O}$ isotope ratios in sea water. A graph similar to that shown in Figure 5 is used.

Environmental science

Roadside soils and vegetation contain high concentrations of lead, mostly derived from petrol additives but less widely known is the relatively high concentration of lead found in upland soils or soils in remote areas of Scotland. It is not clear that such contamination should be derived from petrol additives. It has been recognised for many years that the isotopic composition of lead varies with its source, as in lead derived from natural nuclear fission reactions of uranium and thorium. The ratios of the four lead isotopes, ^{204}Pb ,

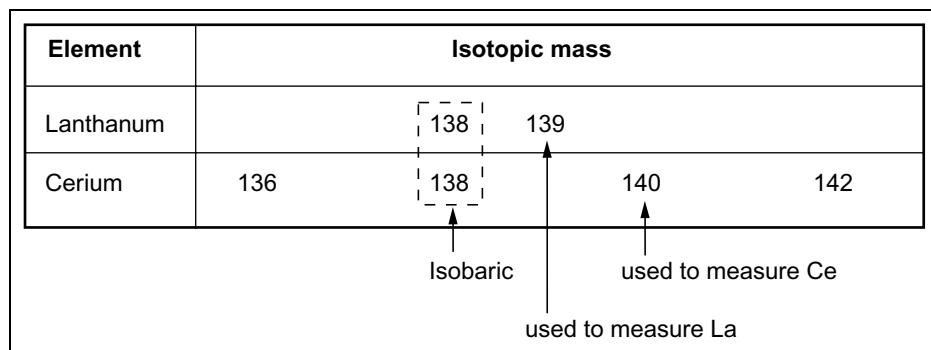


Figure 6 The masses of the naturally occurring isotopes for lanthanum and cerium are shown. For lanthanum, the isotope at 138 is only present in 0.09% natural abundance and is isobaric with ^{138}Ce . For this reason the isotope ^{139}La is used to measure the amount of lanthanum. Similarly, ^{136}Ce and ^{138}Ce are present in low abundance; ^{140}Ce is present in greatest abundance and is used to measure the amount of cerium. Another isotope of cerium, ^{142}Ce , although quite abundant, is isobaric with ^{142}Nd and is therefore not used for measurement.

^{206}Pb , ^{207}Pb and ^{208}Pb , can be measured accurately using an isotopic ratio mass spectrometer that separates and measures simultaneously the four isotopic ion beams for the element. Comparison of lead from different sources has revealed that surface lead from highland areas remote from roads not only had an isotopic distribution different from that present in deeper soil samples but was also different from that provided by lead samples from beside major roads, where contamination was expected from lead additives in petrol. Thus, the lead found in the highland areas remote from roads could not have arisen from the petrol additives. Rainwater over wide areas of the country was found to contain lead corresponding to an input from petrol additives and therefore it was not clear why the highland areas should have had a lead isotope composition different in composition from those of petrol additives.

Rare earth element analysis

The separation of the rare earth elements lanthanum to lutetium provides a significant analytical challenge. Because they are difficult to separate by standard methods, ion exchange resin systems have been developed to differentiate them. This initial ion exchange separation is followed by isotopic analysis on each fraction, using a ratio mass spectrometer. The separation process is time-consuming. Standard mass spectrometric analyses of isotope content without any prior separation are not feasible because the rare earth elements have overlapping isotopes of equal mass (isobaric isotopes). For example, at mass 176, the ^{176}Yb , ^{176}Lu and ^{176}Hf isotopes are isobaric. The problem has been partly resolved by a rapid ion exchange separation of the rare earths into just two fractions, one containing the lower rare earths (LREE; La, Ce, Nd, Sm, Eu) and the other comprising the higher rare earth elements (HREE; Gd, Dy, Er, Yb). The two fractions can be examined separately using an isotope ratio mass spectrometer that has a number of ion collectors, which can simultaneously collect and measure the numbers of ions in the different ion beams. By suitable choice of which isotopes to monitor, it is possible to measure the content of all of the rare earths in a short time. For example, although lanthanum and cerium have isobars at 138, lanthanum has an isotope at 139, which cerium does not have and therefore this mass can be used to estimate lanthanum. Similarly, cerium has an isotope at 140 that is not present in lanthanum (Figure 6). By choosing which isotope to measure, all of the rare earth elements can be analysed accurately and quickly following their ion exchange separation into just two fractions.

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Conclusion Elemental isotopic compositions (isotope ratios) can be used mass spectrometrically firstly in a routine sense to monitor a substance for the presence of different kinds of elements, as with chlorine or platinum, or secondly in a precise sense to examine tiny variations in these ratios, from which important deductions may be made in a wide variety of disciplines.

Table I: Relative abundances of naturally occurring isotopes

IE (eV)	Da/e	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Da/e	
13.6	H	99.9	0.02		100		7.50	92.5		100	19.9	80.1	98.9	1.10	99.6	0.37	99.8	0.04	0.20	100	90.5	H He Li Be B C N O F Ne	
24.6	He																						
5.3	Li																						
9.3	Be																						
8.3	B																						
11.3	C																						
14.5	N																						
13.6	O																						
17.4	F																						
21.6	Ne																						
	Da/e	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	Da/e	
21.6	(Ne)	0.27	9.22	100	79.0	10.0	11.0	100	92.2	4.67	3.10	100	95.0	0.75	4.21	75.8	0.02	24.2	0.06	99.6	93.3	(Ne) Na Mg Al Si P S Cl Ar K Ca	
5.1	Na																						
7.6	Mg																						
6.0	Al																						
8.2	Si																						
10.5	P																						
10.4	S																						
13.0	Cl																						
15.8	Ar																						
4.3	K																						
6.1	Ca																						
	Da/e	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	Da/e	
4.3	(K)	6.73	0.65	0.14	2.09	100	.004		0.19													(K) (Ca)	
6.1	(Ca)																						
6.5	Sc																						
6.8	Ti																						
6.7	V																						
6.8	Cr																						
7.4	Mn																						
7.9	Fe																						
7.9	Co																						
7.6	Ni																						
	Da/e	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	Da/e	
7.6	(Ni)	1.13	3.59	69.2	0.91	30.8	48.6	27.9	4.10	18.8		0.60		27.4	7.80	36.5	100	7.80				(Ni) Cu Zn Ga Ge As Se Br Kr	
7.7	Cu																						
9.4	Zn																						
6.0	Ga																						
7.9	Ge																						
9.8	As																						
9.8	Se																						
11.8	Br																						
14.0	Kr																						
	Da/e	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	Da/e	
9.8	(Se)	49.3	9.20	11.6	11.5	57.0	0.56	72.2	17.3	27.8	9.86	7.00	82.6	100	51.5	11.2	17.2	17.4	2.80	5.52	1.88	(Se) (Br) (Kr)	
11.8	(Br)																						
14.0	(Kr)																						
4.2	Rb																						
5.7	Sr																						
6.4	Y																						
6.8	Zr																						
6.9	Nb																						
7.1	Mo																						
7.3	Tc																						
7.4	Ru																						

Table I: Relative abundances of naturally occurring isotopes (Continued)

IE (eV)	Da/e	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	Da/e
7.4	(Ru)	17.0	31.6	100	18.7																	(Ru)
7.5	Rh			1.02																		Rh
8.3	Pd				11.1	22.3	27.3	51.8	26.5	48.2	11.7											Pd
7.6	Ag						1.2		0.89		12.5	12.8	24.1	12.2	28.7		7.49					Ag
9.0	Cd																					Cd
5.8	In																					In
7.3	Sn																					Sn
8.6	Sb																					Sb
9.0	Te																					Te
	Da/e	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	Da/e
7.3	(Sn)				4.63	5.79																(Sn)
8.6	(Sb)	57.3			42.7																	(Sb)
9.0	(Te)	2.60			0.91	4.82	7.14	19.0	100	31.7		33.8										(Te)
10.5	I																					I
12.1	Xe									0.10	0.09	1.91	26.4	4.10	21.2	26.9	10.4	8.90				Xe
3.9	Cs																					Cs
5.2	Ba																					Ba
5.6	La																					La
5.5	Ce																					Ce
	Da/e	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	Da/e
5.5	(Ce)				11.1																	(Ce)
5.4	Pr	100																				Pr
5.5	Nd		27.1	12.2	23.8	8.30	17.2			5.76		5.64										Nd
5.6	Pm																					Pm
5.6	Sm					3.10			15.0	11.3	13.8	7.40		47.8	26.7	22.7						Sm
5.7	Eu																					Eu
6.1	Gd																					Gd
5.8	Tb																					Tb
5.9	Dy																					Dy
	Da/e	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	Da/e
5.9	(Dy)	18.9	25.5	24.9	28.2				100													(Dy)
6.0	Ho			0.14			1.61		33.6	23.0	26.8		14.9									Ho
6.1	Er																					Er
6.2	Tm																					Tm
6.3	Yb																					Yb
5.4	Lu																					Lu
6.7	Hf																					Hf
7.9	Ta																					Ta
8.0	W																					W
	Da/e	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	Da/e
7.9	(Ta)	99.9																				(Ta)
8.0	(W)		26.3	14.3	30.7		37.4		28.6	62.6												(W)
7.9	Re					0.02			1.58	1.60	13.3	16.1	26.4		37.3	41.0	62.7					Re
8.7	Os																					Os
9.1	Ir																					Ir
9.0	Pt																					Pt
9.2	Au																					Au
10.4	Hg																					Hg
	Da/e	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220	Da/e
10.4	(Hg)	13.2	29.8		6.85			70.5														(Hg)
6.1	Ti				29.5	1.40		24.1	22.1	52.4												Ti
7.4	Pb																					Pb
7.3	Bi																					Bi
	Da/e	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	Da/e
6.1	Th																					Th
5.9	Pa																					Pa
6.1	U																					U

Table 2: Atomic weights of the elements based on the Carbon 12 standard

Symbol	Amu	Abundance	Symbol	Amu	Abundance
H ¹	1.007825037	99.985	Zn ⁶⁴	63.9291454	48.89
H ²	2.014101787	0.015	Zn ⁶⁶	65.9260352	27.81
He ³	3.016029297	0.00013	Zn ⁶⁷	66.9271289	4.11
He ⁴	4.00260325	100.00	Zn ⁶⁸	67.9248458	18.56
Li ⁶	6.0151232	7.52	Zn ⁷⁰	69.9253249	0.62
Li ⁷	7.0160045	92.48	Ga ⁶⁹	68.9255809	60.2
Be ⁹	9.0121825	100.00	Ga ⁷¹	70.9247006	39.8
B ¹⁰	10.0129380	18.98	Ge ⁷⁰	69.9242498	20.52
B ¹¹	11.0093053	81.02	Ge ⁷²	71.9220800	27.43
C ¹²	12.0000000	98.892	Ge ⁷³	72.9234639	7.76
C ¹³	13.003354839	1.108	Ge ⁷⁴	73.9211788	36.54
N ¹⁴	14.003074008	99.635	Ge ⁷⁶	75.9214027	7.76
N ¹⁵	15.000108978	0.365	As ⁷⁵	74.9215955	100.00
O ¹⁶	15.99491464	99.759	Se ⁷⁴	73.9224771	0.96
O ¹⁷	16.9991306	0.037	Se ⁷⁶	75.9192066	9.12
O ¹⁸	17.99915939	0.204	Se ⁷⁷	76.9199077	7.50
F ¹⁹	18.99840325	100.00	Se ⁷⁸	77.9173040	23.61
Ne ²⁰	19.9924391	90.92	Se ⁸⁰	79.9165205	49.96
Ne ²¹	20.9938453	0.257	Se ²²	81.916709	8.84
Ne ²²	21.9913837	8.82	Br ⁷⁹	78.9183361	50.57
Na ²³	22.9897697	100.00	Br ⁸¹	80.916290	49.43
Mg ²⁴	23.9850450	78.60	Kr ⁷⁸	77.920397	0.354
Mg ²⁵	24.9858392	10.11	Kr ⁸⁰	79.916375	2.27
Mg ²⁶	25.9825954	11.29	Kr ⁸²	81.913483	11.56
Al ²⁷	26.9815413	100.00	Kr ⁸³	82.914134	11.55
Si ²⁸	27.9769284	92.18	Kr ⁸⁴	83.9115064	56.90
Si ²⁹	28.9764964	4.71	Kr ⁸⁶	85.910614	17.37
Si ³⁰	29.9737717	3.12	Rb ⁸⁵	84.9117996	72.15
P ³¹	30.9737634	100.00	Rb ⁸⁷	86.9091836	27.85
S ³²	31.9720718	95.018	Sr ⁸⁴	83.913428	0.56
S ³³	32.9714591	0.750	Sr ⁸⁶	85.9092732	9.86
S ³⁴	33.96786774	4.215	Sr ⁸⁷	86.9088902	7.02
S ³⁶	35.9670790	0.107	Sr ⁸⁸	87.9056249	82.56
Cl ³⁵	34.968852729	75.4	Y ⁸⁹	88.9058560	100.00
Cl ³⁷	36.965902624	24.6	Zr ⁹⁰	89.9047080	51.46
Ar ³⁶	35.967545605	0.337	Zr ⁹¹	90.9056442	11.23
Ar ³⁸	37.9627322	0.063	Zr ⁹²	91.9050392	17.11
Ar ⁴⁰	39.9623831	99.600	Zr ⁹⁴	93.9063191	17.40
K ³⁹	38.9637079	93.08	Zr ⁹⁶	95.908272	2.80
K ⁴⁰	39.9639988	0.012	Nb ⁹³	92.9063780	100.00
K ⁴¹	40.9618254	6.91	Mo ⁹²	91.906809	15.05
Ca ⁴⁰	39.9625907	96.92	Mo ⁹⁴	93.9050862	9.35
Ca ⁴²	41.9586218	0.64	Mo ⁹⁵	94.9058379	14.78
Ca ⁴³	42.9587704	0.13	Mo ⁹⁶	95.9046755	16.56
Ca ⁴⁴	43.9554848	2.13	Mo ⁹⁷	96.9060179	9.60
Ca ⁴⁶	45.953689	0.0032	Mo ⁹⁸	97.9054050	24.00
Ca ⁴⁸	47.952532	0.179	Mo ¹⁰⁰	99.907473	9.68
Sc ⁴⁵	44.9559136	100.00	Ru ⁹⁶	95.907596	5.68
Ti ⁴⁶	45.9526327	7.95	Ru ⁹⁸	97.905287	2.22
Ti ⁴⁷	46.9517649	7.75	Ru ⁹⁹	98.9059371	12.81
Ti ⁴⁸	47.9479467	73.45	Ru ¹⁰⁰	99.9042175	12.70
Ti ⁴⁹	48.9478705	5.51	Ru ¹⁰¹	100.9055808	16.98
Ti ⁵⁰	49.9447858	5.34	Ru ¹⁰²	101.9043475	31.34
V ⁵⁰	49.9471613	0.24	Ru ¹⁰⁴	103.905422	18.27
V ⁵¹	50.9439625	99.76	Rh ¹⁰³	102.905503	100.00
Cr ⁵⁰	49.946463	4.31	Pd ¹⁰²	101.905609	0.80
Cr ⁵²	51.9405097	83.76	Pd ¹⁰⁴	103.904026	9.30
Cr ⁵³	52.9406510	9.55	Pd ¹⁰⁵	104.905075	22.60
Cr ⁵⁴	53.9388822	2.38	Pd ¹⁰⁶	105.903475	27.10
Mn ⁵⁵	54.9380463	100.00	Pd ¹⁰⁸	107.903894	26.70
Fe ⁵⁴	53.9396121	5.90	Pd ¹¹⁰	109.905169	13.50
Fe ⁵⁶	55.9349393	91.52	Ag ¹⁰⁷	106.905095	51.35
Fe ⁵⁷	56.9353957	2.25	Ag ¹⁰⁹	108.904754	48.65
Fe ⁵⁸	57.9332778	0.33	Cd ¹⁰⁶	105.906461	1.22
Co ⁵⁹	58.9331978	100.00	Cd ¹⁰⁸	107.904186	0.89
Ni ⁵⁸	57.9353471	67.76	Cd ¹¹⁰	109.903007	12.43
Ni ⁶⁰	59.9307890	26.16	Cd ¹¹¹	110.904182	12.86
Ni ⁶¹	60.9310586	1.25	Cd ¹¹²	111.9027614	23.79
Ni ⁶²	61.9283464	3.66	Cd ¹¹³	112.9044013	12.34
Ni ⁶⁴	63.9279680	1.16	Cd ¹¹⁴	113.9033607	28.81
Cu ⁶³	62.9295992	69.09	Cd ¹¹⁶	115.904758	7.66
Cu ⁶⁵	64.9277924	30.91	In ¹¹³	112.904056	4.16

Table 2: Atomic weights of the elements based on the Carbon 12 standard (Cont'd)

Symbol	Amu	Abundance	Symbol	Amu	Abundance
In 115	114.903875	95.84	Dy 160	159.925203	2.294
Sn 112	111.904823	0.95	Dy 161	160.926939	18.88
Sn 114	113.902781	0.65	Dy 162	161.926805	25.53
Sn 115	114.903341	0.34	Dy 163	162.928737	24.97
Sn 116	115.9017435	14.24	Dy 164	163.929183	28.18
Sn 117	116.9029536	7.57	Ho 165	164.930332	100.00
Sn 118	117.9016066	24.01	Er 162	161.928787	0.136
Sn 119	118.9033102	8.58	Er 164	163.929211	1.56
Sn 120	119.9021990	32.97	Er 166	165.930305	33.41
Sn 122	121.903440	4.71	Er 167	166.932061	22.94
Sn 124	123.905271	5.98	Er 168	167.932383	27.07
Sb 121	120.9038237	57.25	Er 170	169.935476	14.88
Sb 123	122.904222	42.75	Tm 169	168.934225	100.00
Te 120	119.904021	0.089	Yb 168	167.933908	0.140
Te 122	121.903055	2.46	Yb 170	169.934774	3.03
Te 123	122.904278	0.87	Yb 171	170.936338	14.31
Te 124	123.902825	4.61	Yb 172	171.936393	21.82
Te 125	124.904435	6.99	Yb 173	172.938222	16.13
Te 126	125.903310	18.71	Yb 174	173.938873	31.84
Te 128	127.904464	31.79	Yb 176	175.942576	12.73
Te 130	129.906229	34.49	Lu 175	174.940785	97.40
I 127	126.904477	100.00	Lu 176	175.942694	2.60
Xe 124	123.90612	0.096	Hf 174	173.940065	0.199
Xe 126	125.904281	0.090	Hf 176	175.941420	5.23
Xe 128	127.9035308	1.919	Hf 177	176.943233	18.55
Xe 129	128.9047801	26.44	Hf 178	177.943710	27.23
Xe 130	129.9035095	4.08	Hf 179	178.945827	13.79
Xe 131	130.905076	21.18	Hf 180	179.946561	35.07
Xe 132	131.904148	26.89	Ta 180	179.947489	0.0123
Xe 134	133.905395	10.44	Ta 181	180.948014	99.9877
Xe 136	135.907219	8.87	W 180	179.946727	0.126
Cs 133	132.905433	100.00	W 182	181.948225	26.31
Xe 130	129.906277	0.101	W 183	182.950245	14.28
Xe 132	131.905042	0.097	W 184	183.950953	30.64
Xe 134	131.904490	2.42	W 186	185.954377	28.64
Xe 135	134.905668	6.59	Re 185	184.952977	37.07
Xe 136	135.904556	7.81	Re 187	186.955765	62.93
Xe 137	136.905816	11.32	Oa 184	183.952514	0.018
Xe 138	137.905236	71.66	Os 186	185.953852	1.59
La 138	137.907114	0.089	Os 187	186.955762	1.64
La 139	138.906355	99.911	Os 188	187.955850	13.20
Ce 136	135.90714	0.193	Os 189	188.958156	16.10
Ce 138	137.905996	0.250	Os 190	189.958455	26.40
Ce 140	139.905442	88.48	Os 192	191.961487	41.00
Ce 142	141.909249	11.07	Ir 191	190.960603	38.50
Pr 141	140.907657	100.00	Ir 193	192.962942	61.50
Nd 142	141.907731	27.09	Pt 190	189.959937	0.012
Nd 143	142.909823	12.14	Pt 192	191.961049	0.78
Nd 144	143.910096	23.83	Pt 194	193.962679	32.80
Nd 145	144.912582	8.29	Pt 195	194.964785	33.70
Nd 146	145.913126	17.26	Pt 196	195.964947	25.40
Nd 148	147.916901	5.74	Pt 198	197.967879	7.23
Nd 150	149.920900	5.63	Au 197	196.966560	100.00
Sm 144	143.912009	3.16	Hg 196	195.965812	0.146
Sm 147	146.914907	15.07	Hg 198	197.966760	10.02
Sm 148	147.914832	11.27	Hg 199	198.968269	16.84
Sm 149	148.917193	13.84	Hg 200	199.968316	23.13
Sm 150	149.917285	7.47	Hg 201	200.970293	13.22
Sm 152	151.919741	26.63	Hg 202	210.970632	29.80
Sm 154	153.922218	22.53	Hg 204	203.973481	6.85
Eu 151	150.919860	47.77	Tl 203	202.972336	29.50
Eu 153	152.921243	52.23	Tl 206	204.974410	70.50
Gd 152	151.919803	0.20	Pb 204	203.973037	1.37
Gd 154	153.920876	2.15	Pb 206	205.974455	25.15
Gd 155	154.922629	14.73	Pb 207	206.975885	21.11
Gd 156	155.922130	20.47	Pb 208	207.976641	52.38
Gd 157	156.923967	15.68	Bi 209	208.980388	100.00
Gd 158	157.924111	24.87	Th 232	232.03805381	100.00
Gd 160	159.927061	21.90	U 234	234.04094740	0.0058
Tb 159	158.925350	100.0	U 235	235.04392525	0.715
Dy 156	155.924287	0.0524	U 238	238.05078578	99.28
Dy 158	157.924412	0.0902			

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