Mass Spectrometry - part 1

Mass spectrometry is now a hugely diverse subject, and it's growing all the time. There are numerous ways to ionize things, and numerous ways to separate and analyze the ions. It is almost impossible to give a good overview; here I merely give a few highlights from the history of mass spectroscopy, hoping to show how it has developed over a very short period of time.

The origins of mass spectrometry

Towards the end of the 19th century there was a tremendous flurry of building cathode ray tubes. These came in every possible shape and size, and were made not only by physical researchers, but also by instrument companies as demonstration pieces. Cathode rays (obviously) appeared at the cathode and moved towards the anode. You could tell this because if you placed an obstacle in the way (the Maltese cross in the famous Maltese cross tubes), it cast a nice shadow at the anode end of the tube.

But this was a time when new rays were being discovered every few years. It was only natural, since cathode rays were so beautiful, to go looking for anode rays. These appeared, in Goldstein's Canal Ray tubes (1886). They were called canal rays by Goldstein because he cut canals through his cathode, and the rays appeared "overshooting" through the canals.

Fig. 1

Fig. 1 shows a Goldstein tube, which is shown "lit" in Fig. 2. The anode rays, or canal rays, are the purplish glow in the top of the tube. In this case they are protons.

Fig. 2

Cathode rays could be deflected by a magnet, or by an electric field. Initially it seemed that anode rays could not.

In fact, this turned out to be a mistake. Anode rays could be deflected, but only by very big magnets or electric fields. Deflection depends on the mass per charge, since it is the charge that interacts with the magnetic or electric field, and the mass which causes a particle to have inertia, and not move so much as it might (remember $F=ma$)

W. Wien established that anode rays had about 2000 times the mass of cathode rays, so they were 2000 times harder to deflect. This is because they were protons rather than electrons.

The real advances to anode-ray science were made in the lab of J.J. Thomson, at the Cavendish labs in Cambridge. Thomson had discovered the electron in 1897, and although the fundamental unit of charge had not yet been measured (that was left to Millikan in 1909), he knew that there was a universal carrier of negative charge, the electron. Now he wondered if there was a universal carrier of positive charge, and turned his attention to the anode, rather than the cathode ray.

Fig. 3 - Thomson

Thomson's approach was to measure the mass to charge ratio of different positive rays (i.e. using different gases in his tubes), by deflecting them in both electric and magnetic fields. The fields both went in the same direction. Since magnetic fields deflect charged particles sideways to the field, this means that Thomson deflected his rays both upwards and sideways.

The faster a particle was going, the less time it had to get deflected. Since Thomson had a range of speeds of particles, he ended up with a parabola on his target. The beauty of his scheme was that all particles of the same *m/z* ratio would fall on the same parabola.

Although initially he had difficulty finding anything apart from hydrogen, he found that under better vacuum conditions, different gases gave different parabolas.

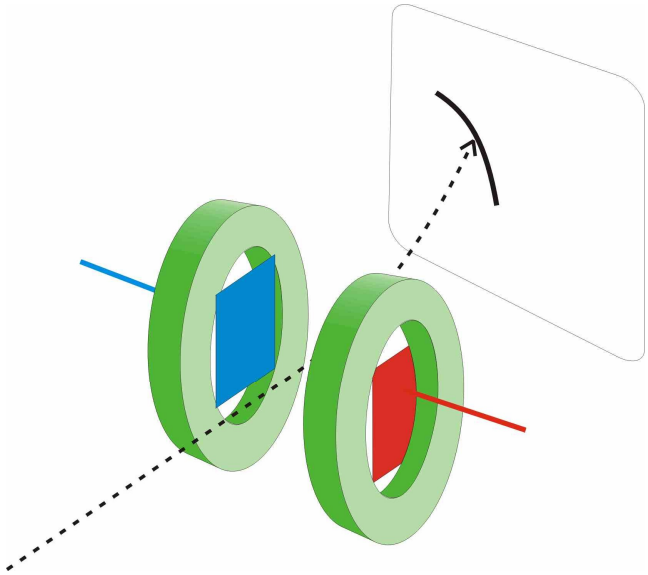


Fig 4 – movement of an ion beam in B/E fields

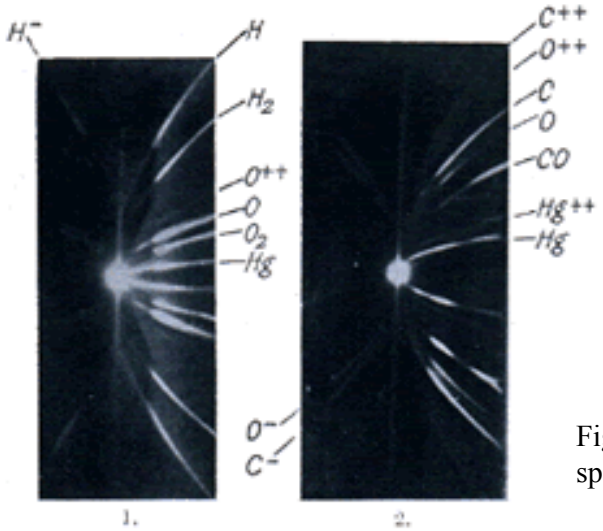


Fig 5 – A typical parabola spectrograph result

Thomson's "parabola spectrograph" produced its most intriguing result with Neon.

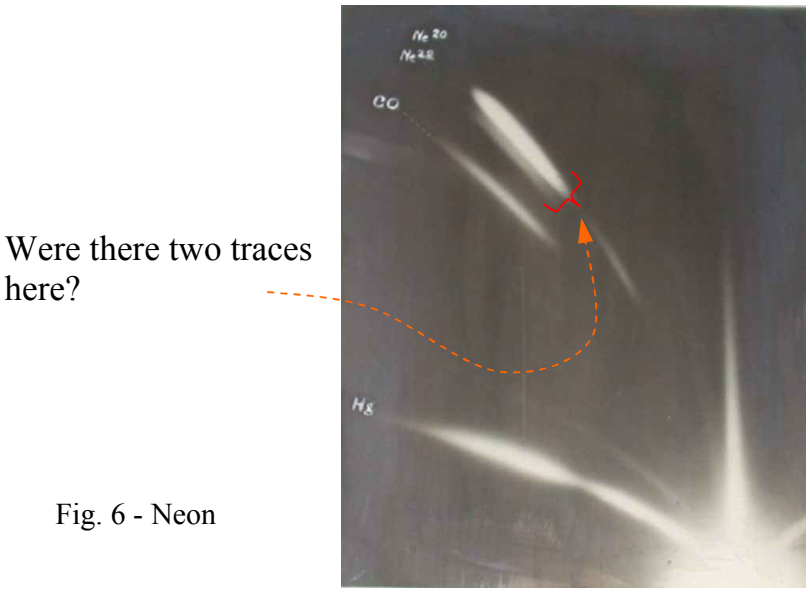


Fig. 6 - Neon

It looked as though there were two parabolas, for 20 and 22. This was the first discovery of a stable (non-radioactive) isotope.

In 1909, Francis Aston joined Thomson, and worked on the development of better mass spectroscopy equipment. His first instrument was used in 1919. He turned the electric field round 90º so that it moved the ions in the opposite direction to the magnetic field, rather than sideways. The advantage was that fast ions were deflected upwards by one, downwards by the other, and slow ions were deflected up rather more by the first, and down rather more by the second. The speed effects cancelled, and both fast ions and slow ions ended up at the same spot. This focussed the ions, and improved resolution:

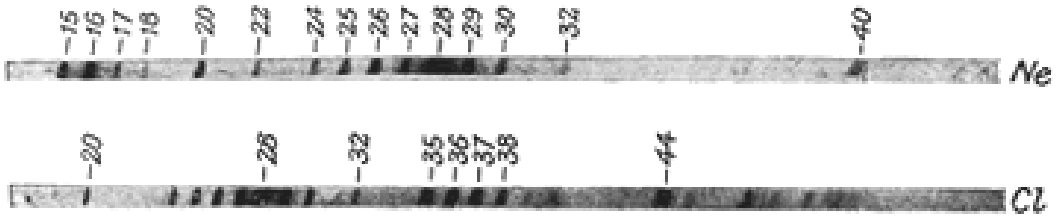


Fig. 7 – Aston's improved spectra

Aston also established the whole-number rule. All spots happened at integer intervals. Originally people thought this meant that each nucleus contained an integral number of protons. It wasn't until Chadwick discovered the neutron in 1932 that people realised the situation was a little more complicated.

Aston worked through a series of three mass spectroscopes, of increasing resolution and sophistication. His lab is shown below, with model No. 3. This was good enough to detect the non-integral mass of hydrogen, an energy difference later attributed to

the lack of nuclear bonding energies in the hydrogen nucleus (where the single proton has nothing to bond with!). It was a practical consequence of Einstein's $e=mc^2$.

Fig. 8 – Aston's lab

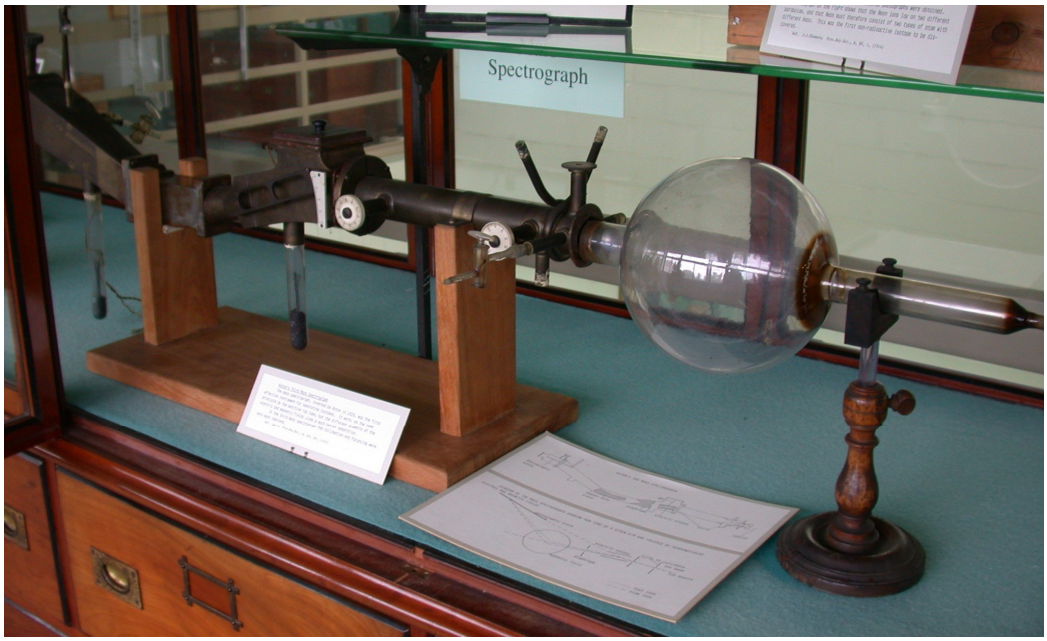


Fig. 9 – Aston's third mass spec tube; obviously the magnets etc. are not included

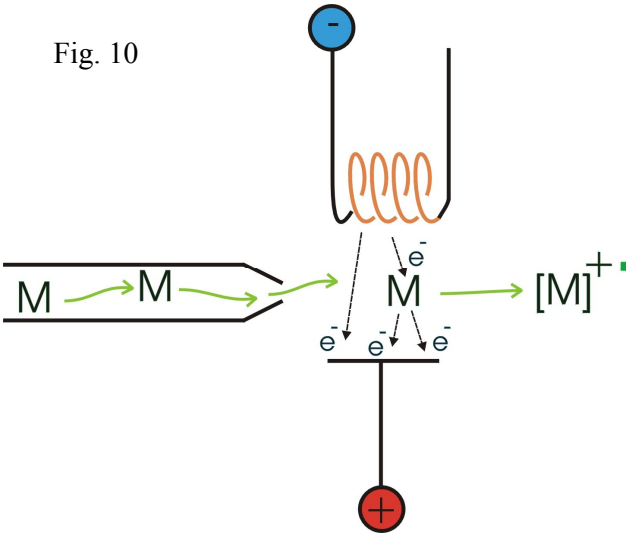
These were the first mass spectrometers that can be recognised as the direct forefathers of the instruments found today. Focussing using electric and magnetic sectors remains the technique learnt at school and university, and used in high resolution instruments. There are now many other sorts of instruments, but always the same stages are found:

1. ionization
2. separating the ions
3. detection of ions

GC-MS and Electron Ionization

Aston's instruments ionized gases, and did so because the stream of electrodes from the cathode was energetic enough to ionize anything in the vicinity. This applies beautifully to gas chromatography, where the gases coming out the end of the column can be led directly into an electron beam. This fact was exploited by Fred McLafferty in 1956.

Fig. 10



The process of ionization involves a high-speed electron (70eV) colliding with a gas molecule. It adds enough energy for one of the molecule's own electrons to escape, leading to a positive, free-radical ion.

Initially GC-MS equipment was outrageously expensive, partly because the ion analyzers were not cheap. Modern GC-MS has been helped hugely by the invention of the very cheap quadrupole analyzer.

The quadrupole mass filter

The first commercial quadrupole instruments were sold in 1968, but the concept of this, and the ion trap (see below) had been developed previously by Steinwedel and Paul, who received Nobel prizes for their work. The quadrupole is a set of 4 poles down the middle of which the ion beam passes:

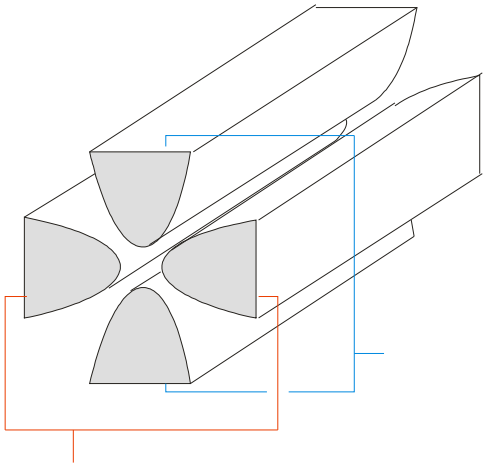


Fig. 11 – a quadrupole

There are two ways to understand the quadrupole. The first is to think about how ions move down it. The opposite poles of the quadrupole are linked, and voltages applied across them. If the voltage is sinusoidal, the ions will be pulled round in little circles; they will spiral down the middle of the poles. Small ions will move further (they have less inertia) in bigger circles. If the voltage is big enough, they will hit a pole, and be lost.

If the voltage is constant, the ions will veer off sideways. If both a constant and an alternating voltage are applied together, summed, then the ions will veer *and* move in circles. It turns out that big ions veer off steadily and are lost, while small ions don't veer because the alternating, circular motion keeps pulling them back into the middle of the poles. It acts like the rifling in a rifle barrel. But small ions do get lost by

circling too wildly. Only ions of just the right size can avoid either veering off, or being dragged into bigger and bigger circles. The quadrupole thus acts as an ion filter, letting only just the right size of ions get all the way to the other end of the rods.

The other way to understand the quadrupole mass filter is to consider a stability diagram of an ion in its field. This is a graph of DC (constant) voltage component and AC (sinusoidal) in which an area is marked, inside which ions are stable (i.e. they won't hit an electrode). The stable area is calculated using the Mathieu equations.

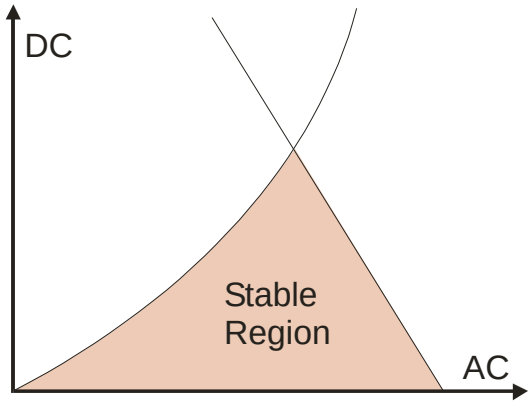


Fig 12 – stability in a quadrupole

The diagram makes sense if you follow the axes; in the absence of AC, any DC voltage at all will cause an ion to veer off and be lost (y-axis). Too much AC will cause an ion to be lost (x-axis). With increasing AC, an increasing DC "veer" voltage is needed to overcome the rifling effect.

Ions of different *m/z* have different stability regions. A typical quadrupole mass spec scans the DC and AC voltages together, along a predefined line in the stability diagram, which just touches the tip of the stable region of each ion. Therefore each ion in turn becomes stable for a brief moment, and is allowed to reach the end of the quadrupole. The mass spec can then detect the ions, and attribute them to mass according to when in the scan they were allowed through. This results in a spectrum as shown lined up with the scan line in Fig. 13.

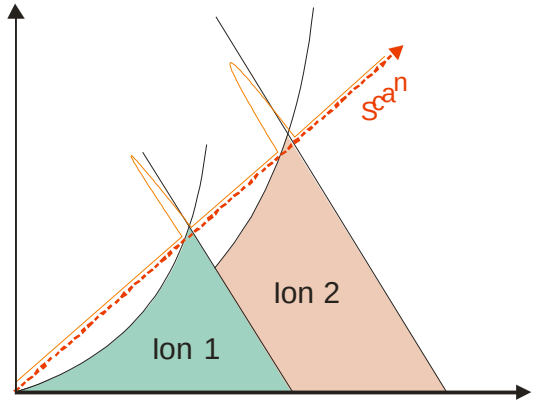


Fig. 13 – scanning a quadrupole

Incidentally, this is why quadrupole instruments are much more sensitive when in "SIM" (single ion monitoring) mode than in scan mode. In SIM mode they sit at a single point in the diagram, and watch the ion of interest continuously, rather than only spotting it briefly every scan.

Electrospray

While gas chromatography interfaced to mass spec quite easily, liquid chromatography was more painful. The reason is that the mass spec needs to operate at a vacuum, and a small amount of liquid (1mL.min^{-1} chromatography flow) can generate a very great deal of gas indeed when evaporated. It is hard to get rid of the LC chromatography flow. Many solutions have been tried over the years, but electrospray has emerged as one of the simplest; it earned its inventor, John Fenn, the Nobel prize in 1988.

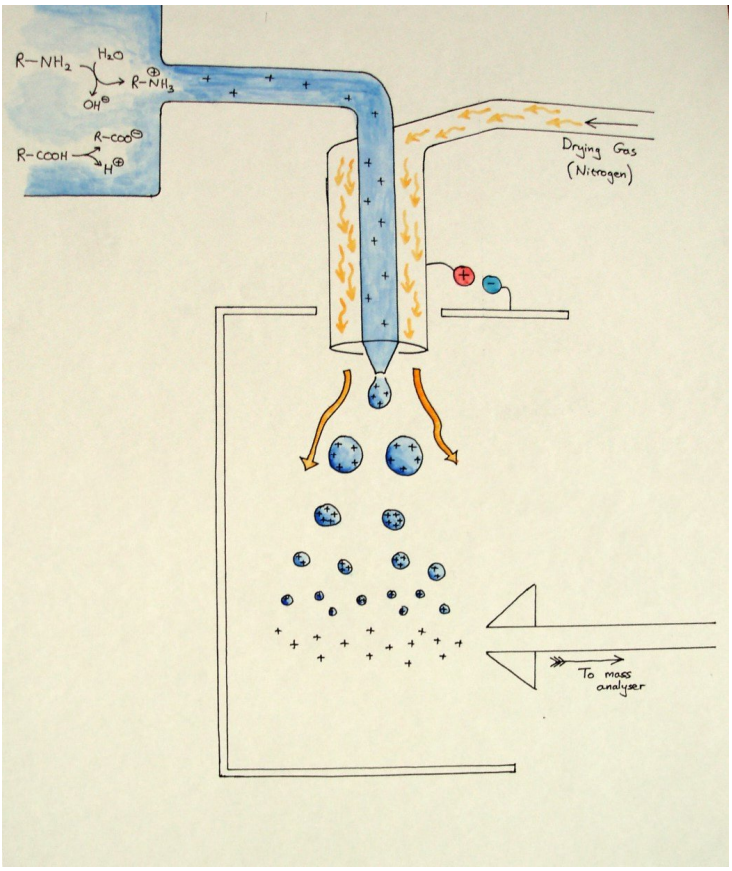


Fig. 14 - electrospray

In electrospray, the LC chromatography flow is squirted through a needle, concentric to another needle carrying an inert gas. This makes a fine spray of droplets. A large voltage is applied between the needle and the area in which the spray appears. There are various theories about what happens next.

The droplets will contain ions in solution. It is possible that some of these are attracted out of the drip by the large electric field. However, the drip will also be shrinking as solvent evaporates in the stream of drying gas. Because of the electric field, like-charges of ions will have collected at the outside of the drip, and will be repelling each other. As the drip shrinks, they are forced closer and closer, and the repulsive force grows, until it is greater than the surface tension of the droplet. The droplet then breaks into several smaller droplets, and the process continues again, all the faster because several small droplets have a greater surface area than one big. In the end there is no solvent left, just ions floating about in an inert atmosphere of drying gas.

This method works provided the solvent forms droplets, and can evaporate, and provided the analyte can form ions in solution (polar things are good; totally non-polar hydrocarbons are hopeless).

Contrasting Electrospray (ESI) and Electron Ionization (EI)

	Electrospray	Electron ionization
The parent ion	A pseudo-molecular ion as found in solution; carboxylic acids form negative ions by loss of H^+; amines form positive ions by gaining H^+ or other positive ions, often Na^+. These are called adducts	A molecular ion which is also a free radical
Fragmentation (see below)	Slight; electrospray is very gentle	Extensive; the full 70eV of original energy has to be got rid of, and it's greater than a bond energy
Size of analyte	Anything goes; Fenn's Nobel prize lecture was about his work on large proteins.	Usually small and volatile
Polarity of analyte	Must be able to form an ion in solution without too much difficulty	Anything goes
Number of charges	Often one, but can be more, especially for big molecules	One

The spectrum below is electrospray of aspirin in negative mode. You should compare it with the NIST library entry (Electron ionization) from the internet. Note that aspirin is a carboxylic acid of neutral mass 180Da, so it forms an ion of 179amu (amu = atomic mass unit; I use this to distinguish between *m/z* values and masses, which are *m*. The aspirin ion is singly charged, so *m/z* = *m*, but in multiply charged ions this isn't the case).

The NIST EI spectrum has a molecular ion peak of 180, the free radical. It is a very small peak.

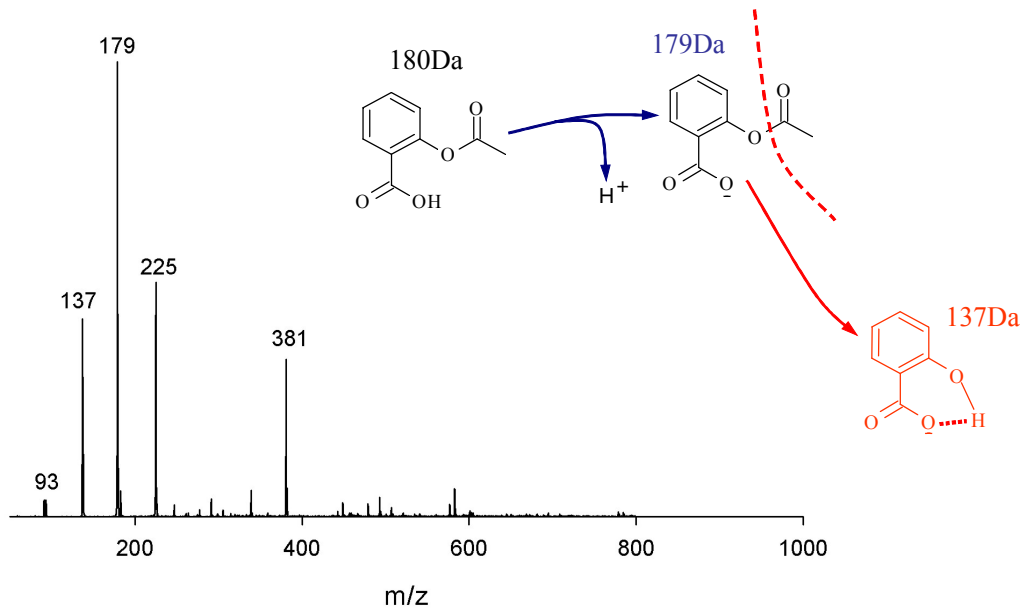


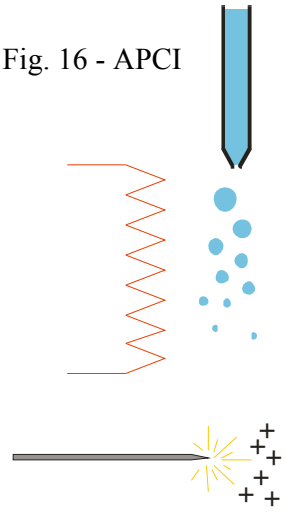
Fig. 15 – electrospray of aspirin

Note that the ESI spectrum also contains the fragments 137amu and 93amu, and a few adducts. 225amu is the formic acid adduct (formate is commonly added to chromatography solvents). Adduct formation is quite normal in ESI. At high concentrations, samples tend to dimerize a little.

Other ways to ionize things

There are many ways to ionize things, but two are worthy of particular note because you will commonly find them provided on typical biological lab instruments.

Fig. 16 - APCI



Atmospheric pressure chemical ionization (APCI) is a complement to electrospray. The sample is sprayed in the same way, but the solvent is dried off by a high temperature heater. It is then ionized by being allowed to drift into a coronal discharge, a spark. This contains lots of highly energetic ionized and radical species, and can ionize things slightly less polar than electrospray can manage. It still can't manage things that have no polar group at all.

Charge transfer chemical ionization, or simply chemical ionization, is found in GC instruments. A small amount of a gas such as methane or ammonia is allowed to trickle into the source. Instead of ionizing the sample directly, the electron beam ionizes the gas, and the gas then ionizes the sample. This allows a much more controlled transfer of energy to the sample, depending on the gas you chose. It allows ionization with a lot less fragmentation, and is useful if you want to find the molecular mass of the sample.

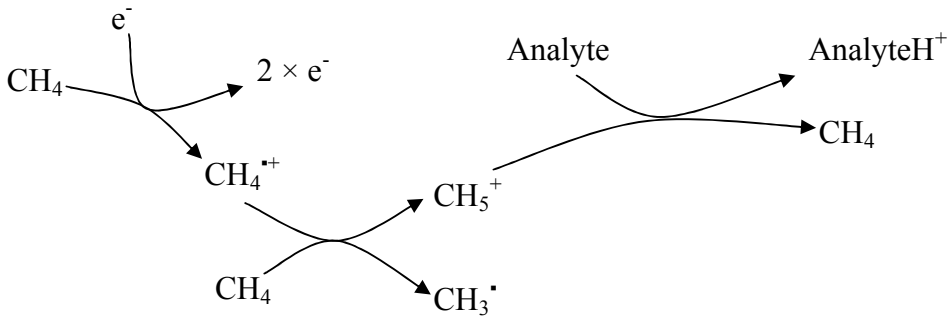


Fig. 17 – charge transfer mechanism for methane as ionizing gas in CI. Note that not all ions become positively charged this way. It is also possible for CH5+ to remove a complete neutral hydrogen from something resulting in CH_4, H_2, and $Analyte^-$.

Acknowledgements

Pictures of Goldstein tubes are by kind permission of Henk Dijkstra, and can be found at his excellent cathode ray tube site:
www.members.chello.nl/~h.dijkstra19/

Pictures from Thomson and Aston's labs and work are by kind permission of the Cavendish labs museum, whose site can be found at:
http://www-outreach.phy.cam.ac.uk/camphys/index.htm