2.2: Instrumentation

Learning Objectives

- Understand how mass spectrometer works
- Learn about the different parts of a mass spectrometer

Principles of Mass Spectrometry

Mass spectrometry (MS) is a powerful characterization technique used for the identification of a wide variety of chemical compounds. At its minimum, MS is merely a tool for determining the molecular weight of the chemical species in a sample. However, with the high resolution obtainable from modern machines, it is possible to determine structural information from the fragments. There are libraries of mass spectra have been compiled which allow rapid identification of most known compounds, including proteins. MS relies on the ability of a compound to be ionized, so the limitations of this technique are when the compound of interest is not readily ionized or if it decomposes upon ionization.

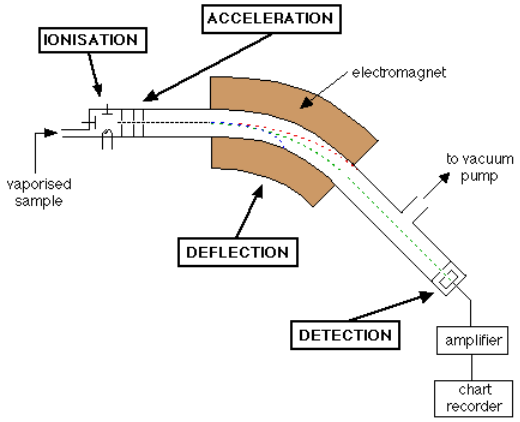
There are many mass spectrometers on the market, but they all have the same basic parts. There is an ionization source, where the molecules are broken into fragments. The mass analyzer is where the ions are separated into their mass/charge ratios before moving to the detector to be observed and counted. Collisions force the fragments to move forward. Consider if something is moving and you subject it to a sideways force, instead of moving in a straight line, it will move in a curve - deflected out of its original path by the sideways force. Suppose you had a cannonball traveling past you and you wanted to deflect it as it went by you. All you've got is a jet of water from a hose-pipe that you can squirt at it. Frankly, its not going to make a lot of difference! Because the cannonball is so heavy, it will hardly be deflected at all from its original course. But suppose instead, you tried to deflect a table tennis ball traveling at the same speed as the cannonball using the same jet of water. Because this ball is so light, you will get a huge deflection. The amount of deflection you will get for a given sideways force depends on the mass of the ball. If you knew the speed of the ball and the size of the force, you could calculate the mass of the ball if you knew what sort of curved path it was deflected through. The less the deflection, the heavier the ball. You can apply exactly the same principle to atomic sized particles.

Atoms can be deflected by magnetic fields - provided the atom is first turned into an ion. Electrically charged particles are affected by a magnetic field although electrically neutral ones aren't.

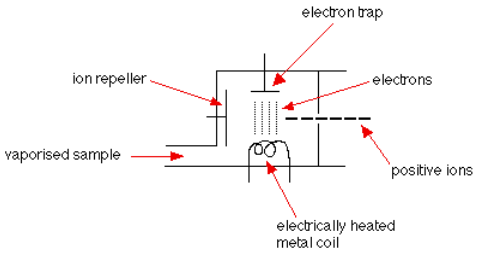
The sequence is :

- **Stage 1: Ionization**: The atom is ionised by knocking one or more electrons off to give a positive ion. This is true even for things which you would normally expect to form negative ions (chlorine, for example) or never form ions at all (argon, for example). Mass spectrometers always work with positive ions.
- **Stage 2: Acceleration:** The ions are accelerated so that they all have the same kinetic energy.
- **Stage 3: Deflection:** The ions are then deflected by a magnetic field according to their masses. The lighter they are, the more they are deflected. The amount of deflection also depends on the number of positive charges on the ion - in other words, on how many electrons were knocked off in the first stage. The more the ion is charged, the more it gets deflected.
- **Stage 4: Detection**: The beam of ions passing through the machine is detected electrically.

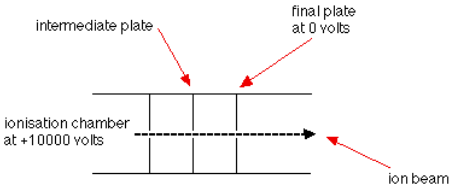
A full diagram of a mass spectrometer is below.



Let's break down the stages in more detail. In order for the ions to have free run of the machine, a vacuum is created in the ionization chamber to avoid air molecules getting in the way. The vaporized sample passes into the ionization chamber. The electrically heated metal coil gives off electrons which are attracted to the electron trap which is a positively charged plate.



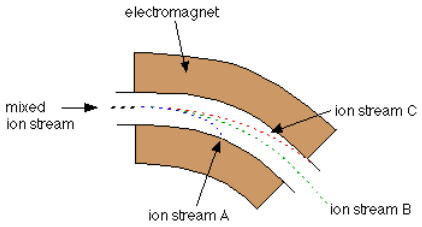
The particles in the sample (atoms or molecules) are therefore bombarded with a stream of electrons, and some of the collisions are energetic enough to knock one or more electrons out of the sample particles to make positive ions. Most of the positive ions formed will carry a charge of +1 because it is much more difficult to remove further electrons from an already positive ion. These positive ions are persuaded out into the rest of the machine by the ion repeller which is another metal plate carrying a slight positive charge. The positive ions are repelled away from the very positive ionization chamber and pass through three slits, the final one of which is at 0 volts. The middle slit carries some intermediate voltage. All the ions are accelerated into a finely focused beam.



Different ions are deflected by the magnetic field by different amounts. The amount of deflection depends on:

- the mass of the ion. Lighter ions are deflected more than heavier ones.
- the charge on the ion. Ions with 2 (or more) positive charges are deflected more than ones with only 1 positive charge.

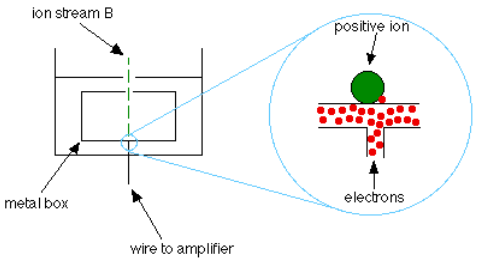
These two factors are combined into the mass/charge ratio. Mass/charge ratio is given the symbol m/z (or sometimes m/e). For example, if an ion had a mass of 28 and a charge of 1+, its mass/charge ratio would be 28. An ion with a mass of 56 and a charge of 2+ would also have a mass/charge ratio of 28. In the diagram below, ion stream A is the most deflected - it will contain ions with the smallest mass/charge ratio. Ion stream C is the least deflected - it contains ions with the greatest mass/charge ratio.



It makes it simpler to talk about this if we assume that the charge on all the ions is 1+. Most of the ions passing through the mass spectrometer will have a charge of 1+, so that the mass/charge ratio will be the same as the mass of the ion. Assuming 1+ ions, stream A has the lightest ions, stream B the next lightest and stream C the heaviest. Lighter ions are going to be more deflected than heavy ones.

Only ion stream B makes it right through the machine to the ion detector. The other ions collide with the walls where they will pick up electrons and be neutralized. Eventually, they get removed from the mass spectrometer by the vacuum pump.

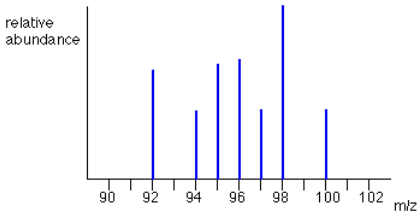
For those ions that make it to the detector, the ion hits the metal box and its charge is neutralized by an electron jumping from the metal on to the ion (diagram below). That leaves a space amongst the electrons in the metal, and the electrons in the wire shuffle along to fill it. A flow of electrons in the wire is detected as an electric current which can be amplified and recorded. The more ions arriving, the greater the current.



How might the other ions be detected - those in streams A and C which have been lost in the machine?

Remember that stream A was most deflected - it has the smallest value of m/z (the lightest ions if the charge is 1+). To bring them on to the detector, you would need to deflect them less - by using a smaller magnetic field (a smaller sideways force). To bring those with a larger m/z value (the heavier ions if the charge is +1) on to the detector you would have to deflect them more by using a larger magnetic field. If you vary the magnetic field, you can bring each ion stream in turn on to the detector to produce a current which is proportional to the number of ions arriving. The mass of each ion being detected is related to the size of the magnetic field used to bring it on to the detector. The machine can be calibrated to record current (which is a measure of the number of ions) against m/z directly. The mass is measured on the ^{12}C scale.

The output from the chart recorder is usually simplified into a "stick diagram". This shows the relative current produced by ions of varying mass/charge ratio. The stick diagram for molybdenum looks like this:



You may find diagrams in which the vertical axis is labeled as either "relative abundance" or "relative intensity". Whichever is used, it means the same thing. The vertical scale is related to the current received by the chart recorder - and so to the number of ions arriving at the detector: the greater the current, the more abundant the ion.

As you will see from the diagram, the commonest ion has a mass/charge ratio of 98. Other ions have mass/charge ratios of 92, 94, 95, 96, 97 and 100. That means that molybdenum consists of 7 different isotopes. Assuming that the ions all have a charge of 1+, that means that the masses of the 7 isotopes on the carbon-12 scale are 92, 94, 95, 96, 97, 98 and 100.

Coupling Mass Spectrometry to Other Instruments

Mass spectrometry is a powerful tool for identification of compounds, and is frequently combined with separation techniques such as liquid or gas chromatography for rapid identification of the compounds within a mixture. Typically, liquid chromatography systems are paired with ESI-quadrupole mass spectrometers to take advantage of the solvated sample. GC-MS systems usually employ electron impact ionization and quadrupole or ion trap mass analyzers to take advantage of the gas-phase molecules and fragmentation libraries associated with EI for rapid identification.

Mass spectrometers are also often coupled in tandem to form MS-MS systems. Typically the first spectrometer utilizes a hard ionization technique to fragment the sample. The fragments are passed on to a second mass analyzer where they may be further fragmented and analyzed. This technique is particularly important for studying large, complex molecules such as proteins.

? Exercise 2.2.1

Does a mass spectrum show the results from just one molecule?

Answer

A mass spectrum does not show the results from one molecule, but from millions of molecules. Because it is displaying results for a population of molecules, more than one mass is shown

Exercise 2.2.1

Where would you expect to find the molecular weight of the molecule?

Answer

A mass spectrum is a bar graph showing the weights of entire molecules as well as smaller pieces of molecules. The entire molecule must have the largest mass, the one farthest to the right, because if a molecule falls into pieces the pieces would be smaller than the whole.

Contributors and Attributions

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