MODULE 2.4

Analysis of trace pollutants in water (continued)

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(Ii) High Performance Liquid Chromatography(HPLC):

HPLC is one of the major techniques of modern analytical chemistry (see fig.1) .It provides high efficiency and high resolution for a wide range of organic compounds and in extensively used in environmental analysis. In this technique the solutes migrate through a column containing a micro particulate stationary phase at rates dependent on their distribution ratios. These are function of the relativel affinities of the solute for the mobile and stationary phases, the elution order depending on the chemical nature of the solutes and the overall polarity of the two phases. Very small particles of the stationary phase is essential for satisfactory chromatographic efficiency and resolution and mobile phase must consequently be pumped through the column at high pressures (200 bar, 3000 psi).The composition of the mobile phase is adjusted to elute all the sample componenets reasonably quickly.After leaving the column the elute passes through the detector which responds to the presence of analytes and develops an electrical signal with a magnitude determine by the amount of analyte passing through the detector.For most purposes a detector is required which will respond to as comprehensive a range of solutes as possible.In this respect a detector based upon the difference in refractive index between the solute and the mobile phase has the widest applicability.However refractive index detectors lack sensitivity relative to others.The most commonly encountered detector type is based upon the absorption of uv radiation by the solute.Few pollutants have sufficient ultra violet absorption for direct detection and so analytes have to be derivatised before analysis.Ultra violet detection is used for

the analyses of N-methyl carbamate, urea and triazine pesticides which belong to second generation pesticides that were developed to replace organic halogen compounds .Preconcentration has to be applied prior to injection.

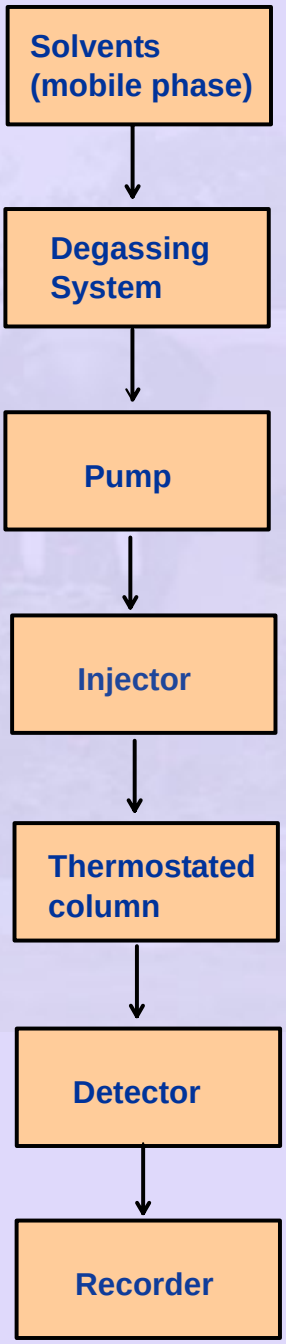

Fig.1 Outline of a HPLC System

The liquid chromotographic methods are more amenable for the analysis of phenols and N-methylthiocarbamates than gas chromatographic methods due to the polarity and thermal lability of these compounds.

Fluorescence detectors are more selective and can be upto three orders of magnitude more sensitive than uv absorbance detectors.In order to monitor very low concentrations,sample preconcentration is needed.Sensitivity can be maximised ,if the detector capable of changing the excitation and detection wavelengths throughout the chromatographic run, since each compound has different optimum settings. For excitation the wavelength used is 270 -300 nm and for detection 330-500 nm. Sometimes even weakly or nonfluorescent compounds can be derivatised to fluorescent compound which can then be detected. For example phenols and N-methylcarbamates which are non fluorescent are converted to fluorescent derivatives and analysed.

In the determination of N-methylcarbamates using HPLC,the eluate from the column is hydrolised with sodium hydroxide at $95^{o}C$ to produce methylamine which is the reacted with O-phthalaldehyde and 2-mercaptoethanol to produce a fluorescent derivative which can be measured at an emission maximum at 418 nm after exciting at 230 nm.A detection limit of approximately 1µg l^{-1} per component for a 400 µg l^{-1} sample injected without preconcentration was obtained.

HPLC methods based on fluorescence detection has also been sucessfully applied to determine trace concentrations of polynuclear aromatic hydrocarbons(PAHs) eg:benzo(a)pyrene;benz (a)anthracene which are highly carcinogenic and produced in trace quantities whenever fossil fuels are

burnt.Typical water extracts contain upto 70 PAHs with a total concentration of about 1 µg l^{-1}.

HPLC methods using conductivity detection is particularly suitable for the determination of anions in determination of most of the common anions.

Low molecular weight carboxylic acids such as formic acid and acetic acid which have similar properties to the inorganic acids can be conveniently be determined by ion-chromatographic methods.Cations including the common metal ions,can also be determined by ion-chromatography.The details of this technique has already been described in detail under chapter 5.2.

Mass spectrometric detection of HPLC effluents has been used for the determination of number of water polutants that cannot be subjected to gas chromatography.

(iii) Analysis Of Metal Ions Present At Trace Levels:

Among the metal ions present in water sodium,potassium,calcium and magnesium are present at levels above mg l^{-1} levels and their determinations has already been discussed in the previous chapter. Of the remaining metal ions, iron, manganese and zinc can sometimes reach towards mg l^{-1} level ,but other metal ions if present will be at µg l^{-1} level concentrations.Of the various instrumental techniques used for the analysis of metal ions in general ,atomic spectroscopy is the most widely used.

Atomic spectrometry includes techniques like (a) flame atomic spectrometry (flame AAS) (b) graphite furnace atomic absorption spectroscopy(flameless AAS) (c)Inductively coupled plasma optical emission spectrometry (1CP-OES) and (d) inductively coupled plasma mass spectrometry (1CP-MS).

Sample Containers and storage:

The sample containers for the storage of metal ions should be made of polythene as they are less likely to contaminate with metal ions than that of glass bottles. Only in the case of mercury ions, the samples should be stored in glass bottles as they react with organic materials. The samples should be slightly acidified to minimise the precipitations of metal ions.

a) Flame Atomic absorption spectrometry:

The technique of atomic absorption is based upon absorption of monochromatic light by a cloud of atoms of the analyte metal. The monochromatic light can be produced by a source composed of the same atoms as those being analysed.The source produces intense electromagnetic radiation with a wavelength exactly the same as that absorbed by the atoms, resulting in extremely high selectivity.The basic components of an atomic absorption instrument is shown in fig 2.

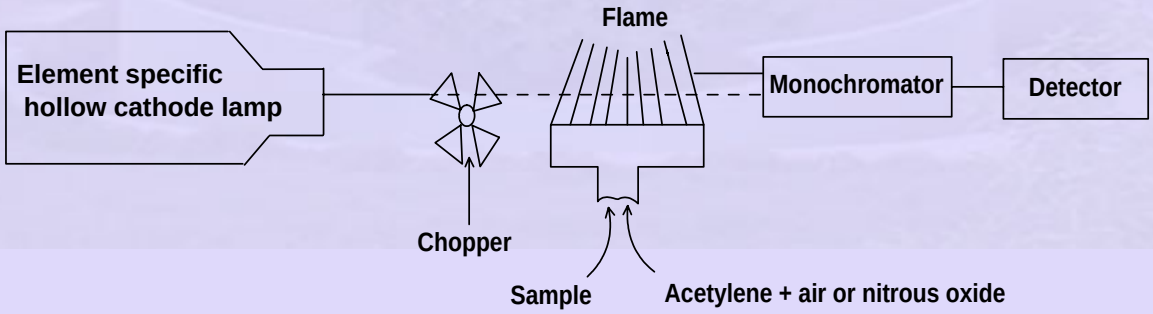

Fig 2 Block diagram for flame atomic absorption spectrometer.

A light beam of the correct wave length to be specific to a particular metal is directed through a flame from the hollow cathode lamp. The sample solution in the form of an aerosol is introduced into the flame. The flame atomises the sample, producing atoms in their ground (lowest) electronic energy state. These

are capable of absorbing radiation from the lamp. The chopper helps in measuring only the intensity of the light absorbed by the ground state atoms in flame. The absorbance observed is related to the concentration. This technique can be applied in general to the concentration range 0 - 5 mg l^{-1}. Some of the advantages of this technique are i) it is rapid ii) simple and routine iii) standard procedures are easily available for all metals. iv) Generally free from interferences, and known interferences can easily be overcome. v) highly sensitive. vi) can be easily automated

For the analysis of magnesium using this technique the samples have to be diluted before analysis. If the technique is used for the analysis of sodium or potassium lower sensitivity absorption lines have to be used in addition to diluting the sample.

Many metals such as Cd,Cr,Co,Ni,Pb,Mn and Ag at low concentrations may be extracted with a chelating agent, such as ammonium pyrrolidine dithiocarbamate (APDC). The metal chelate formed is then extracted with a suitable solvent, such as methyl-isobutyl ketone(MIBK). The MIBK extract is then aspirated directly into the flame. The increase in the sensitivity is above that which is expected from a simple concentration factor. This is due to the increased aspiration rate resulting from the lower viscosity of the organic solvent and easier atomization in comparison with water.

The major drawbacks of the flame AAS technique are that it is less sensitive since the nebulisation efficiency is very small and large amount of samples are necessary for the analysis.These difficulties are overcome in flameless atomic absorption spectroscopy.

b) Flameless atomic absorption spectroscopy:

It is a graphite furnace with an electrothermal atomization device that consists of a hollow graphite cylinder placed so that the light beam passes through it. A small sample of upto 100 µl is inserted in the tube through a hole on the top. An electric current is passed through the tube to heat it- gently at first to dry the sample, then rapidly to vaporise and excite the metal analyte. The absorption of metal atoms in the hollow portion of the tube is measured and recorded as a spike-shaped signal. In the furnace mode of AAS the temperature produced is much higher than that obtained from flame. Thus, better sensitivity and much lower detection limit of metals are obtained. Another advantage of the graphite furnace technique over the conventional flame is that the former requires a smaller volume of sample. On the otherhand, a disadvantage of the furnace technique is that because of its high sensitivity, interference from other substances present in the sample is often manifested. Such interference can be removed or reduced by adding a matrix modifier to the sample or by correcting for background absorbance. Flame mode is simple and suitable for determining metal ions at relatively higher concentrations above 1 mg l^{-1}).

c) Determination of tin, lead and metalloids by hydride generation method:

Elements such as tin ,lead ,arsenic and selenium are converted into their hydrides in an HCl medium by treatment with sodium borohydride.The hydride formed are purged into the atomiser with argon or nitrogen for conversion into gas-phase atoms.The calibration standards for these elements should also be converted into their hydrides in the same manner.

d) Cold vapour method for measuring mercury :

Inorganic mercury salts can be chemically reduced using tin (ii) chloride or sodium tetrahydroborate. The elemental mercury produced is then swept by a stream on nitrogen or air into a gas cuvette for absorption measurement in a modified spectrometer.

e) Inductively coupled plasma atomic emission spectroscopy (ICP-AES):

ICP-AES offers certain advantages over flame AAS and flameless AAS. It provides simultaneous or sequential multi element analysis in a single analysis.Thus several metals can be determined rapidly in a sample in a simple run. It also provides a long linear range for metal ions over several orders of magnitude whereas the linear range of metal ions in AAS is very limited.

In this technique the sample is atomised in a plasma flame at 6000-10,000 K and the emission spectrum is monitored. Sixty or more elements can be at once determined at preset wavelengths.The problem of spectral overlap can be overcome by proper choice of analytical wavelength based on freedom from interference and sensitivity.

f) Inductively coupled plasma mass spectrometry (ICP-MS):

This technique consists of inductively coupled plasma with a quadrupole mass spectrometer. ICP of high energy generates charged ions from the atoms of the elements present in the sample. The ions generated are directed on to a mass spectrometer, separated, and measured according to their mass- to -charge ratio. The method is highly sensitive and the detection limits of some metals may be 100 times lower than that obtained by graphite furnace AAS

technique but is still sufficient to determine trace metal ions at below 1 mgl^{-1} in aqueous samples.

g) Anodic stripping voltammetry:

Anodic stripping voltammetry is designed to measure trace amounts of analyte by preconcentrating them onto a suitable electrode. The experiment has two stages. The sample is electrolysed onto a hanging mercury drop, or a mercury film deposited on a carbon electrode. The reduced species (ie.the metal) is then oxidised out of the film by making the electrode increasingly anodic. A peak appears on the current potential plot. A typical current potential plot for the analysis of Cu, Pb and Cd is shown in figure 3.

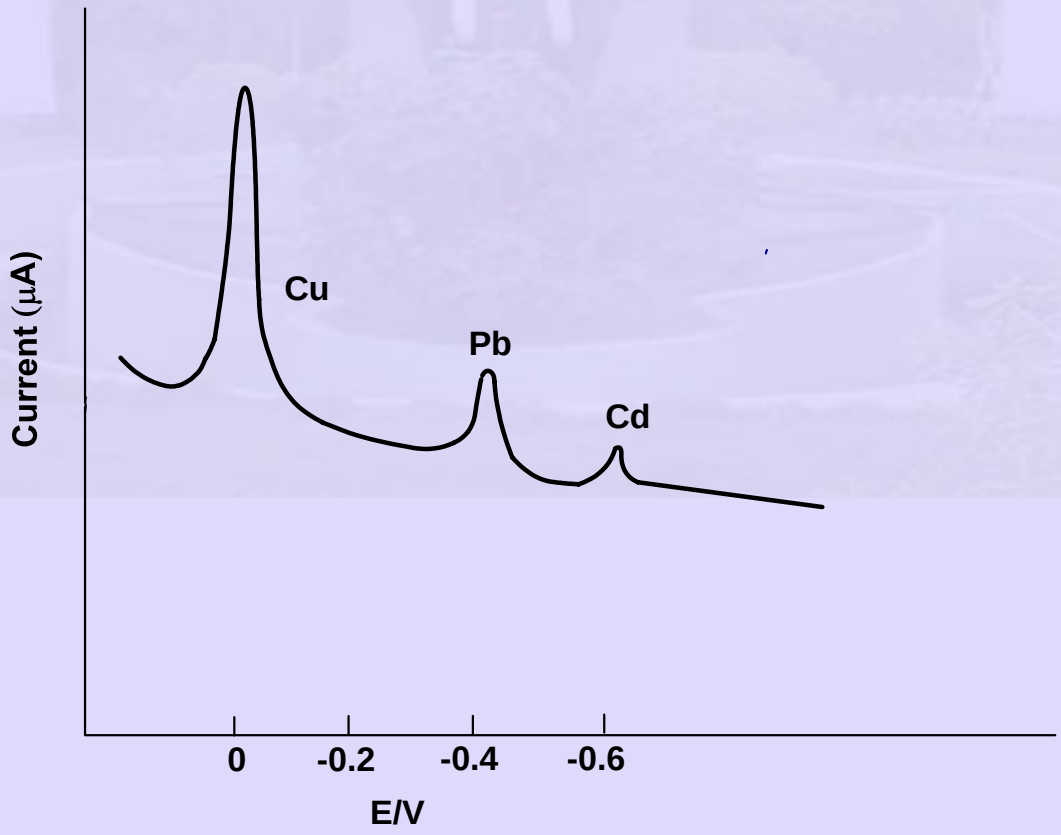

Fig 3 Anodic stripping voltammogram of Cu, Pb & Cd

The peak potential at which an active species is oxidised is characterstic of that species ,and is close to its half-wave potential. The height of the peak in the curve is proportional to the concentration of the metal. Applications of anodic stripping voltammetry are chiefly for the determination of trace amounts of amalgan-forming metals.The preconcentration stage allows determination in the concentration range 10^{-6} to 10^{-8} M.

This method determines only free metal ions in solution and to some extent loosely associated complexes. If the total metal content is required, sample pretreatment is necessary. This is done by either acidifying the sample or destroying the potential complexing agents by uv radiation. The individual concentration of free and complexed metal ions can be determined by performing the analysis with and without pretreatment by this method. This type of individual determination of free and complexed metal ions is not possible in atomic spectrometry. However this is not a rapid method.

(iv) Chelation ion liquid chromatography:

This technique finds use in environmental analysis where atomic spectroscopy is not ideal such as the following. For example

(i) Extraction techniques are often necessary when complex samples are analysed by AAS in order to remove interfering components. This extends the time taken to perform an analysis considerably.

(ii) In the analysis of mixtures of uncommon elements by AAS, additional or unsuspected elements will not be detected. With the correct choice of column and eluent these would be seen as additional peaks in a liquid chromatographic analysis.

(iii) In the analysis of different chemical forms of the ion in which a metal can be found in the environment, ion chromatography can sometimes separate and quantify chemical forms. Atomic spectroscopy is unable distinguish the different species. Even when comparing with atomic emission techniques liquid chromatographic methods have advantages.

For example sequential plasma emission spectrometers detect a limited number of elements and unsuspected elements may still be missed. Different chemical forms are not distinguished. Emission techniques are, however, more rapid for multi-element analyses.

Chromatographic methods using both dedicated ion chromatographs and conventional HPLC have been developed.The most sensitive method for transition metals in complex mixtures using a dedicated chromatograph is known as 'chelation ion chromatography' .

The selectivity of ordinary cation-exchange resins for various metals ions is somewhat limited.However if a suitable chelating functional group is built into a polymeric resin, it often is possible to take up only a small group of metal ions. Other chelating resins may complex a large group of metal ions, but additional selectivity is attained through pH control. Trace amounts of complexed metal ions may be concentrated from a large sample onto a very short column. Subsequent elution by acid breaks up the metal chelates and gives much more concentrated solution of the metal ions for further analysis. A column packed with a chelating resin may be used to separate sample metal ions based on the differences in the strengths of their chelates. Many types of chelating resins have been synthesised such as iminodiaceticacid (IDA), amidoxine etc.

A high concentration of chelating groups on the chelating resin results in more complete complexation of metal ions from solution, and can also cause resin to retain metal ions from a more acidic sample.This necessitates the use of more concentrated acid solution to break up the complex and thereby desolve metal ions from the resin. The presence of excess acid may complicate the determination of sample ions for subsequent analysis by ion-chromatography.

An additional complication is that two kinds of metal ion uptake can occur with iminodiacetic acid (IDA) chelating resins. The desired kind of uptake involves chelating of metal ions with nitrogen and carboxyl groups of the IDA ligands. The other type is simple ion exchange of cations that are electrostatically attracted to the negatively charged carboxylate groups. This simple ion exchange can take up a significant amount of Na^+ or other unwanted cations, particularly if the resin contains a high concentration of IDA groups.

On balance, the best choice of an IDA resin might be one with moderately low capacity (approximately 0.5m equivalent/g for example) for retaining metal ions by chelation.

For chromatographic separation it is important to use an efficient resin with the chelating groups readily accessible so that the chelation of the metal ion is not sterically inhibited. The equilibrium between a divalent metal ion (M^{2+}) and a chelating resin (RL^-H^+) may be written:

$$M^{2+} + 2RL^-H^+ \Leftrightarrow (RL)_2M + 2H^+$$

An acidic eluent is used to control this equilbrium so that the retention factor of the sample metal ion is in the desired range. Increase in H^+ concentration in the

eluent weakens the chelates and speeds the elution.Separations of different metal ions will occur due to differences in the equilibrium constants.

A second way to separate metal ions on a chelating resin column is to use a complexing agent (E^-) , such as oxalate, tartrate, at a fixed pH. Here a second equilibrium will come into play:

$$(RL)_2M + 2E^- \Leftrightarrow 2RL^- + ME_2$$

Now the retention factor will be influenced by the type and concentration of E^- in the eluent.

A number of disolvent metal ions were effectively separated using these chelating ion-exchange resins. A silica based resin with chemically bonded amidoxine groups was used for the chromatographic separation of transition metal ions (fig.4). Five transition elements were separated using 5mM sodium oxalate at pH 3.6 as eluent. Post-column detection was employed with 0.5 mM 4-(2 pyridylazo) resorcinol (PAR) in 3M ammonia and 1M acetic acid as the colour-forming reagent.

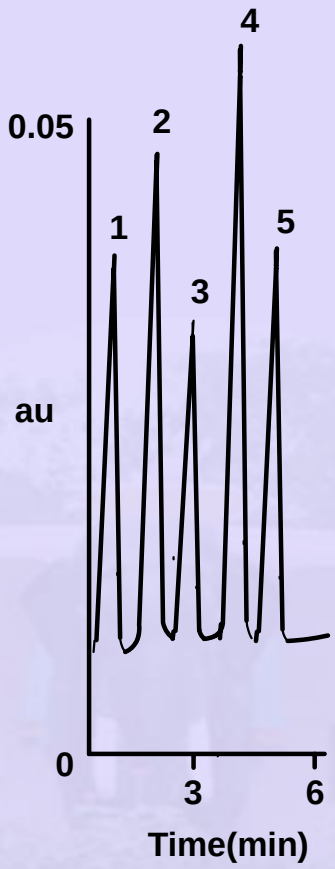

Fig.4 Separation of transition metals with amidoxine column with sodium oxalate as eluent, 5mM sodium oxalate (pH 3.6); Peaks: 1=Cd^{2+}; 2=Co^{2+}; 3=Zn^{2+}; 4=Cu^{2+}; 5=Ni^{2+}

v) Speciation of Chromium by ion chromatography:

Chromium finds its way into the environment through industrial wastes from electroplating sludge, tannery wastes, the manufacture of corrosion inhibitors, and municipal sewage sludge.Cr(III) is essential to human nutrition. Cr(III) is less toxic and less mobile in the environment than Cr(VI)• It would appear that $Cr_2O_7^{2-}$ ion can enter the cells via routes which permit entry of the similarly sized SO_4^{2-} ion. Such a route would not be possible for the positively charged Cr^{3+} ion.

The basis of many of the separations has been to convert Cr(III) to an anion by adding a complexing agent. Cr(VI) is already an anion (usually CrO_4^{2-})and hence, anion chromatography can be used to separate mixtures.

Pyridine dicarboxylic acid (PDCA) was used as a complexing agent for Cr^{3+} to form anion $[Cr(PCDA)_2^-]$. The sample pH is critical to separation. Optimum pH is 6.8.Both Cr(III) and Cr(VI) can be detected by uv-vis detection with diphenyl carbrazide as a post column reagent.

Mass spectrometric detector for GC for the determination of ultratrace levels of (ng l^{-1}) polychlorinated organic compounds:

At the lowest levels of detection, one area of concern is polychlorinated dibenzodioxins (PCDDs) (e.g 2,3,7,8 - tetrachlorodibenzo-p-dioxin and polychlorinated dibenzofurans (PCDFs) (eg. 2,3,7,8 tetrachlorinated compounds). These compounds are formed during the combustion of organic material containing chlorine. An additional property of these PCDDs and PCDFs is their strong binding ability to organic material in soils. The solubilities of these dioxins in water is very low and their bioconcentration factor is very high and their toxicity is acute. Hence there is a need to determine these constituents present in such low concentrations in water and soils. GC/MS has been used for their determination.

Mass spectrometry:

A block diagram of a typical molecular-mass spectrometer is shown in fig.5. Sample molecules enter the mass spectrometer through an inlet system. In the case of GC, the sample is in the form of vapour and the inlet must interface between the atmospheric pressure GC system and the low pressure

(10^{-5} to 10^{-8} torr) mass spectrometer system. An elaborate vacuum system is needed to maintain the low pressure. In the mass spectrometer, the sample molecules enter

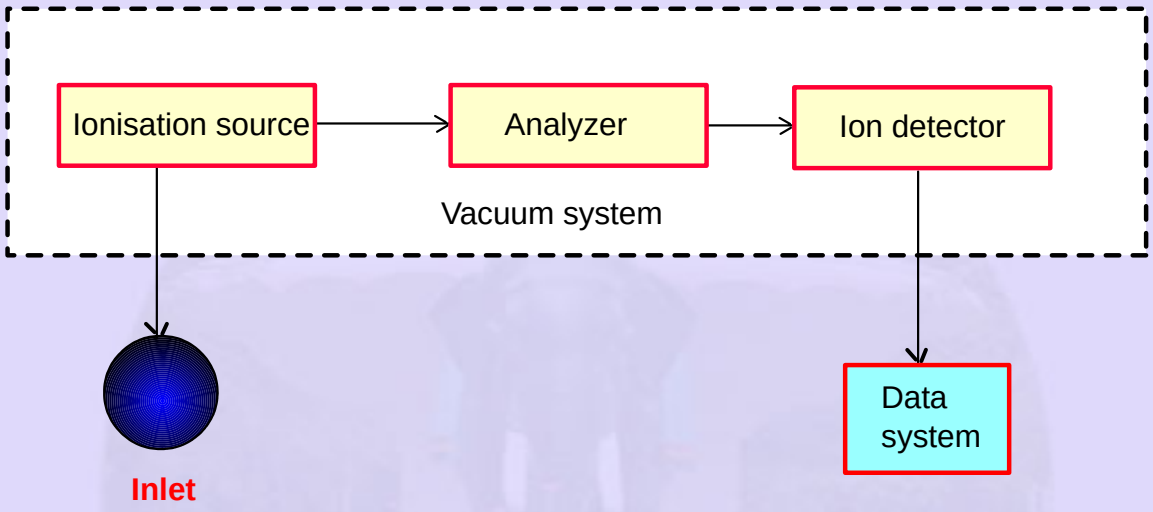

Fig.5 Out line of a mass spectrometer

an ionisation source, which ionises the sample. The ionisation sources for molecular spectrometry are energetic enough to break chemical bonds in the sample molecules, but not so energetic as to decompose the sample molecules into their constituent atoms, as is done in atomic mass spectrometry. The ionisation sources in GC/MS produce fragments, which can also be ionised. Hence leaving the ion source are ions of the sample molecules, called **molecular ions,** fragment ions, and unionised molecules. The unchanged molecules and fragments are normally pumped out of the ion source by vacuum pumps used to produce low pressure environment. Next the analyser sorts the ions according to their m/z values, just as in atomic mass spectrometry. The separated ions are then detected, and a plot of ion intensity versus m/z value is produced by the data system.

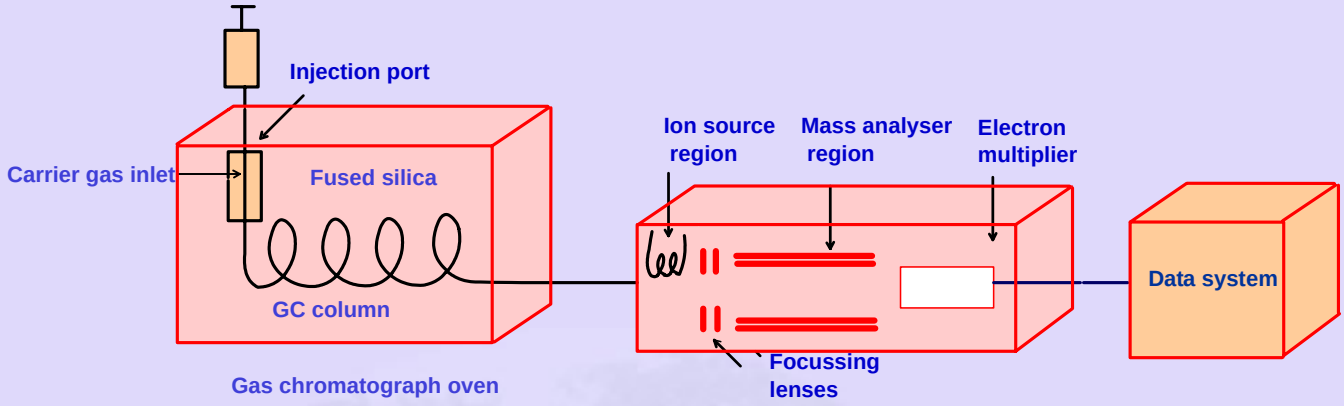

Fig.6 A Schematic of a GC/MS System

The schematic of a complete GC/MS instrument shown in fig.6. Here the sample is injected into the capillary GC, and the effluent enters the inlet of a quadrupole mass spectrometer. The molecules are then fragmented and ionised by the source are mass analysed, and are detected by the electron multiplier.

In GC/MS, the mass spectrometer scans the masses repeatedly every second during a chromatographic experiment. The data can be analysed by the data system in several different ways. First the ions abundances in each spectrum can be summed up and plotted as a function of time to give a **total-ion chromatogram**. This plot is similar to conventional chromatogram. One can also display the mass spectrum at a particular time during chromatogram to identify the species that is eluting at that time. Finally, one can select a single m/z value and monitor it throughout the chromatographic experiment, which is called **selected ion monitoring**.

Toxic organic pollutants like polychlorinated dibenzo-p.dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), p.p' DDT, dieldrin, lindane, malathion, 2,4,5,2',5' polychlorinated biphenyls and 2,3,7,8-tetrachlorodibenzo-p-dioxin etc

are analysed using GC/MS. The analytical determinations of these organic pollutants which are sometimes at ng l^{-1} concentrations involve four main stages and they are (i) extraction of the analyte (ii) separation and interfering compounds (iii) concentration (iv) and analytical separation and determination by gas chromatography using mass spectrometric detector.

The potential interferences in the chromatogram can be detected if fragments are monitored at two or more mass/charge ratios, known as selective-ion monitoring as mentioned previously. When applied to dioxin analysis, the technique makes use of naturally occuring chlorine being found as an approximate 3:1 mixture of ^{35}Cl and ^{37}Cl isotopes. Their intensities should be in the ratio 3:1. If the fragment is not detected at both m/z values then the assumption that the fragment contains chlorine is wrong. If the relative intensities are not 3:1 and if it is certain that there is just one chlorine in the fragment, then this would suggest that there is interference from a second ion which coincidentally has m/z value identical with that of one of the ions. If the fragment contains more than one chlorine atom, the pattern will be complex, but still predictable and easily recognisable with experience.