GAS CHROMATOGRAPHY - MASS SPECTROMETRY (GC-MS)

CHROMATOGRAPHY is a physical method of separation in which the components to b arated are distributed between two phases. One of these phases is STATIONARY (Solid, alumina, silica, graphite, cellulose, paper or Liquid such as SE-30, c OF1, OV-101 made stationary on an inert support), the other one MOBILE (usuall solvents and inert gases (carrier gas), H_e, H_2, N_2, Ar). Depending upon the of the two phases different forms of chromatography such as column, thin-layer gas liquid, or liquid liquid - chromatography result.

In gas chromatography volatile substances are separated and analysed in t vapour phase (hence the name vapour phase chromatography). The instrument de to perform this operation consists essentially of the following components:-

(a) a gas cylinder to provide mobile phase,

(b) pressure regulator to maintain a constant flow of the carrier gas,

(c) gas or liquid sample injector block through which the sample is introduced means of a microsyringe.

(d) a packed glass or metal column - where the mixture is partitioned and sepa

(e) an oven chamber with temperature programming facility,

(f) Detector (such as FID, ECD, MS, UV, IR, Ar) and

(g) a recorder.

Prior to the analysis, an appropriate column is selected (polar columns for pol compounds, non-polar columns for analysis of non-polar compounds), conditioned heating at the required temperature while maintaining a constant flow of carri (30-60 ml/min.). If the sample proves to be relatively non-volatile on a pre ary run, then it is treated with a suitable reagent so that a more volatile de suitable for analysis is produced. A small amount of the reaction product is introduced on to the column by means of a micro-syringe (0.1 to 5 µl of the sol The column outlet is connected to a device capable of detecting the presence of the COLLECTOR. The outcoming signal is plotted as a function of time (Chromat There are more than 40 types of detectors in use, the most common being the Fl Ionization Detector, Electron Capture Detector, Mass Spectrometer, and Thermal Conductivity Detector.

The retention time - the time elapsed between the emergence of solvent and of t maximum and the area of the peak ($A = \int_{I}^{II} Sdt$ cm^2 or Asec or Vsec) are used in t qualitative analysis. For a given set of conditions, the retention times are acteristic of compounds and remain constant. Two compounds are said to be res if they appear as two distinct peaks on the chromatogram.

Preparation of derivatives

Highly polar, low volatile or thermally unstable samples are not suitable direct injection on to a GC. In such cases, blocking of reactive sites in the molecule by non-polar groups reduces the polarity and thus modifies the physica

properties of the parent compound.

Usually the following reactions are carried out on a microscale:-

Substrate	Reagent	Derivative
Acids	diazomethane	Methyl esters
Alcohols	oxidising agents	Aldehydes/Ketones
	BSA	Trimethylsilyl ethers (TMS)
Amino-acids	BSA	TMS derivatives
Sterols	BSA	" "
Carbohydrates	BSA	" "
Peptides	LAH/$SOCl_2$/LAH	Amine derivatives etc.

GC-MS

GC when used alone provides a means of separating mixtures on the basis of retent time data. As many variables such as type and condition of the column, flow ra temperature influence the retention times, identification of components on this alone becomes inconclusive. Whereas when a GC is coupled to an MS by means of separator, the resulting instrument inherits all the advantages of the two instr but eliminates the disadvantages, and becomes ultrasensitive. In this collecti samples are introduced in the GC, separated and purified and presented to the ma spectrometer one at a time - which are then detected and identified by the mass spectrometer. Unambiguous identification of samples present in very low levels to 10^{-14} g) thus becomes possible.

A MASS SPECTROMETER is a very sensitive instrument operating under high vacuum, d to analyse sub-microgram quantities of a compound wherein it is bombarded with su energy (20-70 eV) to cause fragmentation, and the resulting positively charged ic are accelerated (3-8 kV) into a magnetic field (H), analysed according to their m to charge ratio (m/e), and are recorded on a photosensitive paper as a series of vertical lines. The resulting record of ion abundance against mass/charge cons a fragmentation pattern. This pattern provides information regarding the accura of the molecular ion (M^+), structural information and mode of fragmentation of th molecule under electron impact. This information is sufficient to identify the

The equation which describes the path of the ion trajectory can be written a follows:

$$m/e = \frac{R^2H^2}{2V}$$ where R is the radius of curvature.

For a given instrument R is constant and for given values of H and V, only the i particular m/e ratio are focussed on the detector. The entire mass spectrum can

obtained by scanning either H or V.

A high vacuum system, ion source, inlet systems, electrostatic and magnet analysers, a collector (electron multiplier), UV-recorder and the essential electronic assembly form the integral parts of a mass spectrometer.