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COLUMN CHROMATOGRAPHY

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Column Chromatography is a routinely carried out technique which is adaptable to all the major types of chromatography such as ion exchange, exclusion and affinity chromatography.

Columns

The column is made up of glass and poly-acrylic plastic .
Size of column usually have a diameter of 2-70 mm and length of 15-150 cm.
Choice of the column of a particular dimension is dictated primarily by the amount of sample.
Glass column have sintered glass disc at the bottom to support the stationary phase.
Some column with thermo-state jacket are used for the prevention of harmful of temperature fluctuations.
The columns are provided with an inlet and an outlet for eluting solvent to enter the column and for effluent from the column respectively.

Packing the Column

The column is fitted in the upright position and its bottom is sealed with glass-wool.
The column is filled to about one third its height with the mobile phase. A thick suspension ,called slurry, is gently poured into the column with its outlet closed.
The upper part of column is stirred to ensure even packing and avoid air bubbles. The slurry is usually added till ¾ th of the column is full. The outlet is now opened and the column is stabilized by washing it with mobile phase . A filter paper or nylon gauze is then placed on the surface of the column to prevent disturbance.

Introduction of Sample

It is necessary that the sample to be applied reaches the surface of the column below the top layer of the solvent . This can be achieved by sucking the top layer of solvent out and then carefully pipetting the sample on the column surface. Solvent is then added to the column to a height of 5-10 cm. The column is then connected to a suitable reservoir which contains more solvent .
Another alternative method is to mix the sample with sucrose or ficoll to a concentrate of about 1% to increase sample density. The dye bromo-phenol blue can be used in place of sucrose.

Column development

Continuous passage of a suitable eluent (mobile phase) through the packed column separates the components of the sample applied to the column. This process is known as column development.

When a single solvent is used as an eluent during development, the process is known as isocratic separation. For satisfactory resolution the PH , ionic strength or polarity of the eluent is changed with respect to time. This process where the composition of the mobile phase is changed giving rise to a gradient is known as gradient elution. This technique leads to a better resolution of the sample components.

Flow Rate

Flow rate Fc , is expressed as the volume of mobile phase per unit time. The flow rates used differ with respect to the nature of the sample and the nature of the stationary phase . However, commonly used flow rates fall between 30-120 ml / hour cm2.

Column packing also influences flow rate . It is very important to pack the column optimally to obtain a good flow rate.

Analysis and collecting of effluent

The effluent , as it emerges from the column outlet is analyzed. The properties of a particular compound ,such as ultraviolet absorption, colour or fluorescence are exploited in its analysis effluent . The modern approach, is to continuously monitor the effluent coming out of the column. To monitoring equipment is programmed to read the inherent property of the desired compound such as the ultraviolet absorption or radioactivity.

For example if the desired sample component is a protein , the monitoring equipment may be a UV monitor and may be programmed to read absorption at 280 nm.

A wide variety of monitoring equipments are fluorescence detectors , polarimeters , voltameters , refractometric detectors and conductivity detectors.

The electrical signal generated by the detector due to the property of the compound which it is reading is displayed on a chart recorder.
The area under the peak is proportional to the amount of the compound present. This area may be determined by measuring the height of the peak and its width at half the height.

Fractions in small volumes may be collected in different tubes in order to keep the column resolved component separate from each other. If the separation has been satisfactory , a particular component will be distributed in relatively few number of tubes. The content of these tubes, which contain the same component , can then be pooled together for further study.

A range of automatic fraction collectors are available commercially. They are design to collect a definite volume of the effluent in each tube before a new tube is placed in position automatically.

Recent innovations in fraction collecting systems have led to production of fraction collectors which are connected to the monitor in a way that the signal for the fraction collector is derived from the monitor signal.

Column Efficiency

A given column can be divided into a number of identical segments such that one equilibrium distribution takes place on each of these segments. Each of these segments is called a theoretical plate.

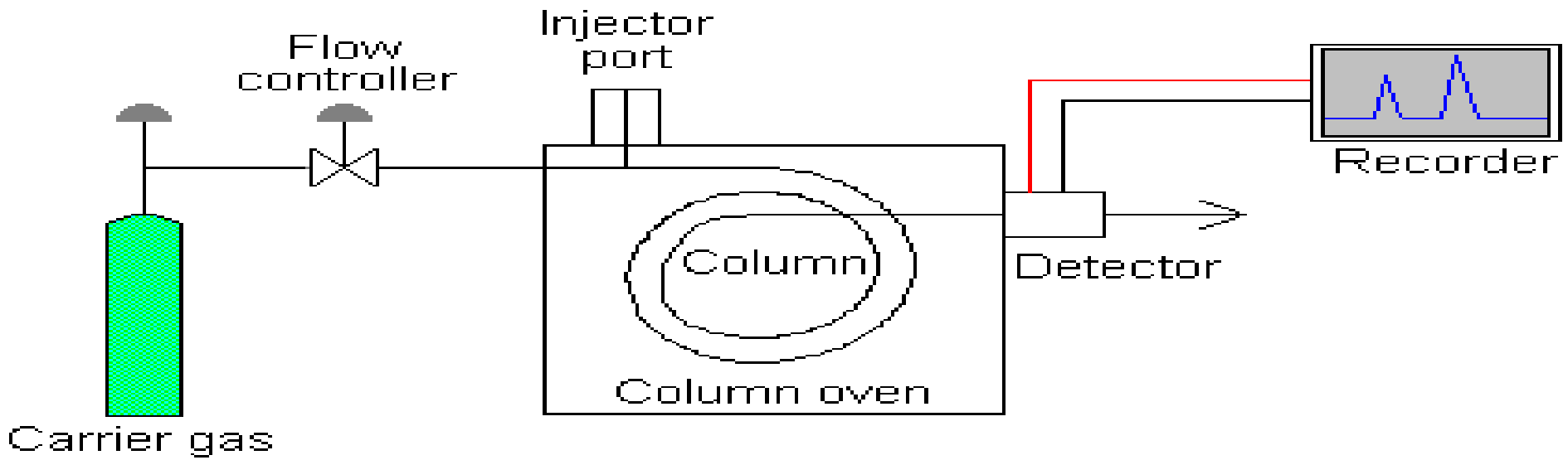
Thus the number of equilibrations taking place in a column is equal to the number of plates that the column possesses. If two solute components have very small difference in their partition coefficients they will need a large number of equilibrations before they can be completely resolved each other.
Thus the column which has a larger number of plates will be better suited for resolving these components whereas a column which has small number of plates will not be as efficient. Therefore column efficiency is directly related to the number of plates it possesses.

We can increase the number of plates without increasing the length of the column . This may be done by making a better column and optimizing other factors which affect the solute interaction with the two phases such as flow rate , temperature , method of sample introduction etc.

This optimization of column development conditions would lead to increased number of equilibrations taking place thereby increasing the number of theoretical plates in the column.

Gas Liquid Chromatography

It is a form of column chromatography
It involves a sample being vapourized and injected on to the head of the chromatographic column. The sample is transported through the column by the flow of inert, gaseous mobile phase. The column itself contains a liquid stationary phase which is adsorbed on to the surface of an inert solid.



Instrumental components

Carrier gas

The carrier gas must be chemically inert. Commonly used gases include nitrogen, helium, argon, and carbon dioxide. The choice of carrier gas is often dependent upon the type of detector which is used. The carrier gas system also contains a molecular sieve to remove water and other impurities

Sample injection port

For optimum column efficiency, the sample should not be too large, and should be introduced on to the column as a "plug" of vapour - slow injection of large samples causes band broadening and loss of resolution.
The most common injection method is where a microsyringe is used to inject sample through a rubber septum into a flash vapourizer port at the head of the column.
The temperature of the sample port is usually about 50°C higher than the boiling point of the least volatile component of the sample.

For packed columns, sample size ranges from tenths of a microliter up to 20 microliters.

Capillary columns, on the other hand, need much less sample, typically around 10^{-3} mL .

For capillary GC, split/splitless injection is used. The injector can be used in one of the two modes ; split or splitless .

The injector contains a heated chamber containing a glass liner into which the sample is injected through the septum. The carrier gas enters the chamber and can leave by three routes (when the injector is in split mode).

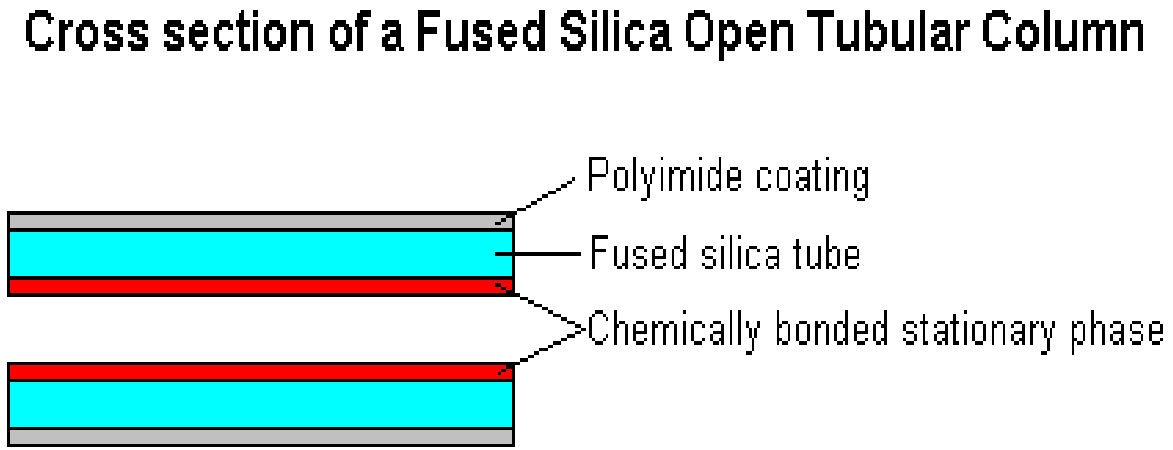
The sample vapourizes to form a mixture of carrier gas, vapourized solvent and vapourized solutes. A proportion of this mixture passes on to the column , but most exits through the split outlet. The septum purge outlet prevents septum bleed components from entering the column.

Columns

There are two general types of column, *packed* and *capillary* (also known as *open tubular*). Packed columns contain a finely divided, inert, solid support material (commonly based on *diatomaceous earth*) coated with liquid stationary phase. Most packed columns are 1.5 – 10 m in length and have an internal diameter of 2 – 4 mm.

Capillary columns have an internal diameter of a few tenths of a millimeter. They can be one of the two types; *wall-coated open tubular* (WCOT) or *support-coated open tubular* (SCOT).

Wall-coated columns consist of a capillary tube whose walls are coated with liquid stationary phase. In support-coated columns, the inner wall of the capillary is lined with a thin layer of support material such as diatomaceous earth, on to which the stationary phase has been adsorbed. SCOT columns are generally less efficient than WCOT columns. Both types of capillary column are more efficient than packed columns.



These have much thinner walls than the glass capillary columns, and are given strength by the polyimide coating. These columns are flexible and can be wound into coils. They have the advantages of physical strength, flexibility and low reactivity.

Column temperature

For precise work, column temperature must be controlled to within tenths of a degree. The optimum column temperature is dependent upon the boiling point of the sample. As a rule of thumb, a temperature slightly above the average boiling point of the sample results in an elution time of 2 - 30 minutes. Minimal temperatures give good resolution, but increase elution times. If a sample has a wide boiling range, then temperature programming can be useful. The column temperature is increased (either continuously or in steps) as separation proceeds.

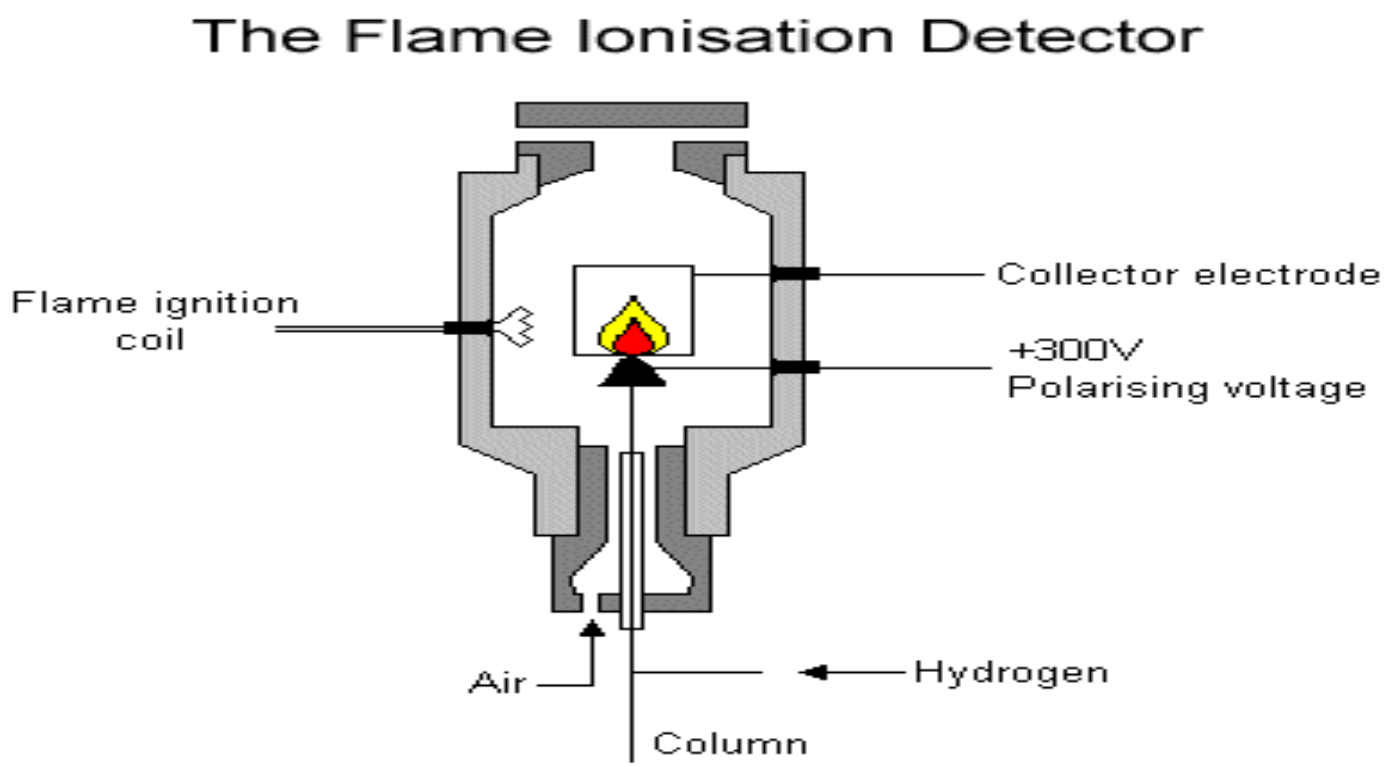
Detectors

There are many detectors which can be used in gas chromatography. Different detectors will give different types of selectivity.

A *non-selective* detector responds to all compounds except the carrier gas, a *selective detector* responds to a range of compounds with a common physical or chemical property and a *specific detector* responds to a single chemical compound.

Detectors can also be grouped into *concentration dependant detectors* and *mass flow dependant detectors*. The signal from a concentration dependant detector is related to the concentration of solute in the detector, and does not usually destroy the sample Dilution of with make-up gas will lower the detectors response. Mass flow dependant detectors usually destroy the sample, and the signal is related to the rate at which solute molecules enter the detector. The response of a mass flow dependant detector is unaffected by make-up gas.

The effluent from the column is mixed with hydrogen and air, and ignited. Organic compounds burning in the flame produce ions and electrons which can conduct electricity through the flame. A large electrical potential is applied at the burner tip, and a collector electrode is located above the flame. The current resulting from the pyrolysis of any organic compounds is measured. FIDs are mass sensitive rather than concentration sensitive; this gives the advantage that changes in mobile phase flow rate do not affect the detector's response. The FID is a useful general detector for the analysis of organic compounds; it has high sensitivity, a large linear response range, and low noise. It is also robust and easy to use, but unfortunately, it destroys the sample.



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