Lecture
10- 9

Chromatography

Prof.Dr.Mohie Sharaf El Din

CHROMATOGRAPHY

CLASSIFICATION OF CHROMATOGRAPHY

A - Based on physical means (Form)

1 - column 2 - planner (TLC , paper chr.)

B - Based on types of equilibrium

i-Adsorption (solid stationary phase)

ii-partition (liquid stationary phase)

iii-ion exchange

iv-size exclusion (gel filtration)

C - Based on type of mobile phase

1 - liquid chr.(LC) 2 - gas chr.(GC)

D - Based on type of stationary phase

1-normal phase 2 - reverse phase 3 - bonded phase

Introduction

- **Most of the chemical analysis are selective , few are specific ,therefore it is very important step in many analytical procedures is the separation of the analyte from the interference**
- **. Chromatography is the most widely used means of performing analytical separation .**
- **Column chromatography was first invented for separation of plants pigments by passing solution of these compounds through column of glass filled with calcium carbonate , the pigments were separated as colored bands (The name " chroma = color , graphien = to write) .**

PRINCIPLES FOR CHROMATOGRAPHIC METHODS

- **Chromatography is taken now to refer generally to the separation of components in a sample by distribution of the components between two phases , one that is stationary (Stationary Phase) and one that move (Mobile Phase) .**
- **All types of chromatography are based on the phenomenon that each component in a mixture ordinarily interacts with its environment differently from all other components under the same conditions .**
- **A common feature of all chromatographic methods is that the separation takes place on a boundary between two phases (solid , liquid , or gas in every combination) .**

- **Chromatographic separation of solutions takes place at the interface between a solid and a liquid , or between two liquids . The separation of gases takes place at the interface of a solid or liquid phase and a gaseous phase .**

- **A column or layer of some material usually serve as the solid phase and depending on its properties the separation takes place according to the following four principles :**

Principles of separation

1 - The material may have a large adsorptive capacity and the separation is based on differing adsorption coefficients of the substances separated between the solid stationary phase and the liquid mobile phase *(Adsorption Chromatography)* .

2 - An inert material may hold a solvent by sorption ,i.e., it retains one liquid phase on its surface forming a stationary phase and the separation takes place on the bases of differing partition coefficients of the separated substances between the stationary phase and the liquid mobile phase *(Partition Chromatography)*

3 - The material may be capable of exchanging its own ions for ions present in a polar mobile liquid (usually water or alcohol) and the separation is based on differing affinity of the separated substances between the mobile liquid (either acidic or basic mobile counter ions) and ion exchange resin (*Ion Exchange Chromatography*) .

4 - *Size Exclusion Chromatography* , in which molecules are separated based on their size by passing through a porous structure stationary phase .

- **Chromatography can be classified according to the form of the stationary phase into *Column Chromatography* in which a solid or liquid stationary phase is packed in a tube or column and *Planner Chromatography* in which the stationary phase is in the form of a sheet or other flat surface including Paper Chromatography , Thin Layer chromatography and Electrophoresis .**

- **Chromatography may also be classified according to the nature of the mobile phase into *Liquid Chromatography* where the mobile phase is liquid and *Gas Chromatography* where the mobile phase is gas .**

CLASSIFICATION OF CHROMATOGRAPHY

A - Based on physical means (Form)

1 – column 2 - planner (TLC , paper chr.)

B - Based on types of equilibrium

i-Adsorption (solid stationary phase)

ii-partition (liquid stationary phase)

iii-ion exchange

iv-size exclusion (gel filtration)

C - Based on type of mobile phase

1 - liquid chr.(LC) 2 - gas chr.(GC)

D - Based on type of stationary phase

1-normal phase 2 - reverse phase

3 - bonded phase

THIN LAYER CHROMATOGRAPHY

- **Thin Layer Chromatography TLC in which the stationary phase is a thin layer of finely divided adsorbent supported on a glass or aluminum plate or plastic strip .**
- **Any of the solids used in column chromatography can be used , provided a suitable binder can be found for good adherence to the plate .**

- **Stationary Phases for TLC**
- **The stationary phase consists of a finely divided powder (particle size 5 - 50 um). Typically 0.1-0.3 mm in uniform thickness .**
- **It can be an adsorbent , an ion exchange , or a molecular sieve , or it can serve as the support for a liquid film .**
- **The most commonly used stationary phases are adsorbents . *Silica gel* . *alumina* and powdered *cellulose* are the most popular . Silica gel and Alumina particles contain hydroxyl group on their surface which will form hydrogen bond with polar molecules . Alumina is preferred for separation of weakly polar compounds , but silica gel is preferred for polar compounds such as amino acids and sugars .**

Magnesium silicate , calcium silicate and activated charcoal **may also be used as adsorbents . Adsorbents are usually activated by heating .**

- **Thin film liquid stationary phase can be prepared for separation by liquid -liquid partition chromatography .**
- **The film , commonly *water* , is supported on materials such as silica gel or diatomaceous earth , as in column chromatography .**
- **Either silica gel or diatomaceous earth may be silanized to convert the surfaces to non polar methyl groups for *reversed phase TLC***

- **Ion exchange resin are available in particle size 40-80 um ,suitable for preparing thin layer plates .**
- **Examples are *Domex 50 W* strong acid cation exchange and *Dowex 1* strong base anion exchange resin .**

- **Size exclusion thin layers can be prepared from *sephadex* superfine . The capillary action through these molecules sieves is much slower than with most other thin layers , typically 1 - 2 cm/hour , and so development takes 8 - 10 hours, compared to about 30 minutes for other stationary phases .**

Mobile Phases for TLC

- **The selection of the developing solvent depends on the stationary phase used .**
- **The eluting power of solvents increases in the order of their polarilies (e.g. from hexane to acetone to alcohol to water) .**
- **A single solvent , or at most two or three solvents , should be used whenever possible .This may result in varying *Rf values* depending on how far the spots are allowed to move up the plate.**
- **The developing solvents must be of high purity . The presence of small amounts of water or other impurities can produce irreproducible *chromatograms* .**

- **Developing the chromatogram**

TLC is very simple form that is used widely for qualitative identification , although quantitative analysis can be done .

- **A sample (dilute solution in volatile nonpolar solvent ;hexane , petrolium ether ; ether) is spotted onto thin layer plate with a micropipette , and the plate is developed by placing the bottom of the plate (but not the sample spots) in a suitable solvent placed at the bottom of certain TLC Jar (tank) .**
- **The solvent is drown up the plate by capillary action , and the sample components move up the plate surface at different rates , depending on the their solubility and their degree of retention by the thin layer plate .**
- **Following development , the individual solute spots are noted or are made visible by treatment with a reagent that form a colored derivative .**

- **The spots will generally move at a certain fraction of the rate at which the solvent moves , and they are characterized by the Rf value**

$$Rf = \frac{\text{distance solute moves}}{\text{distance solvent front moves}}$$

- **The development taking from 10 minutes to one hour ,and is reproducible .**

- Methods of developing the chromatogram:

1 – ascending

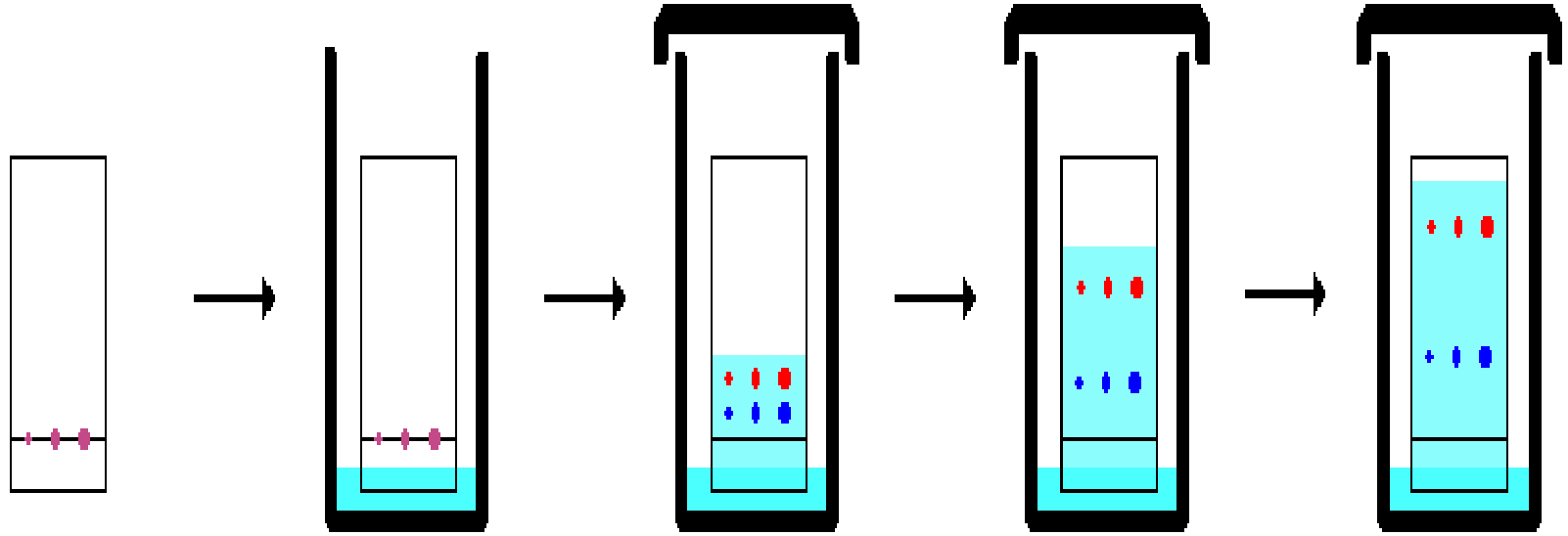
2 – descending

3 – horizontal

4 – two dimentional

TlC plate development sequence:

a mixture of a red and blue compound is separated as the plate develops, i.e. as the light blue solvent moves up the plate.



Detection of the spots

A- Physical methods :

1 - when the separated substances are colored (can be easily detected as shown).

2 - **Thin layer plates or sheets are commercially available which incorporate a fluorescent dye in the powdered adsorbent . When under UV light , dark spots appears where sample spots occur due to quenching of the plate fluorescence .**

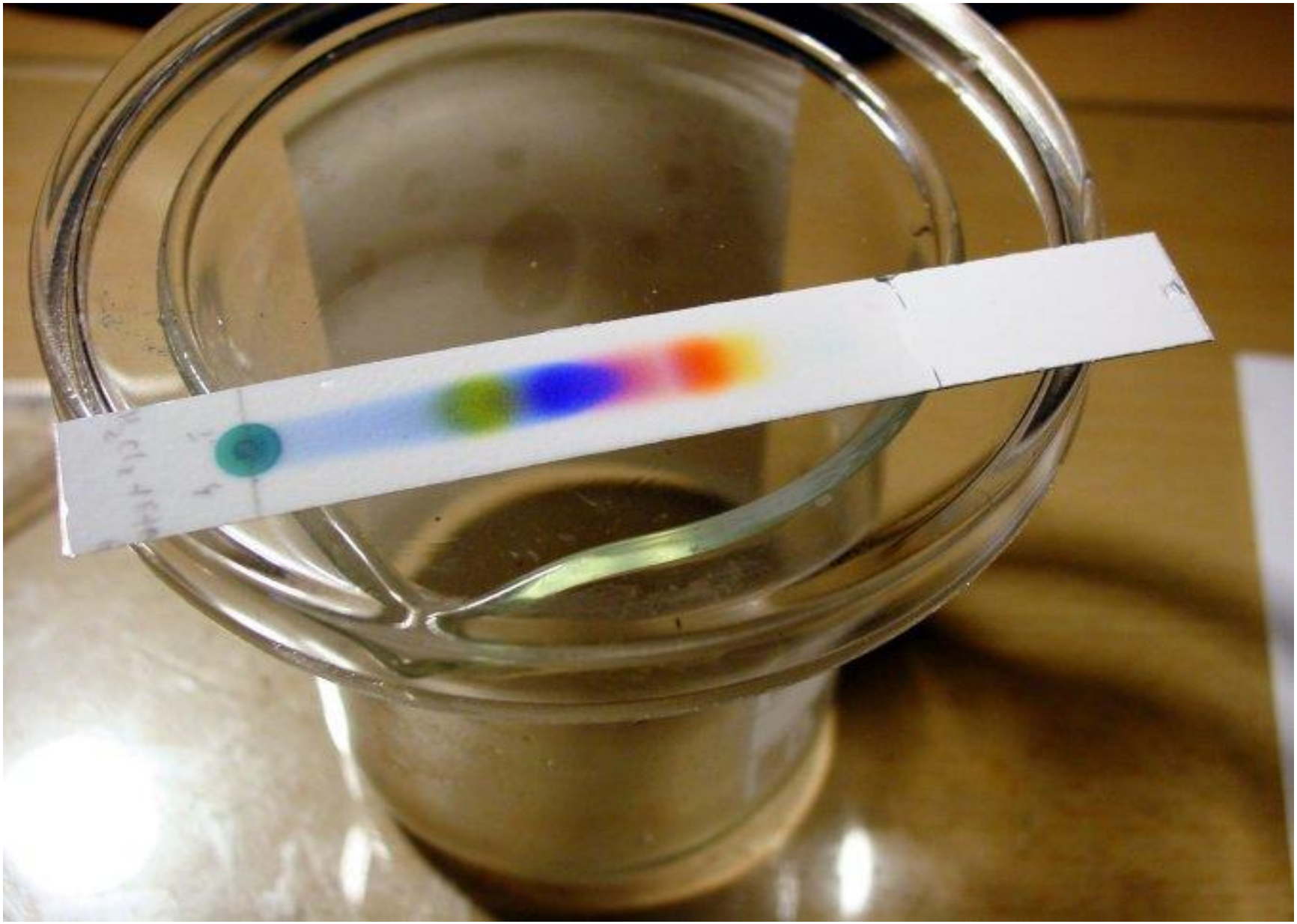
B – Chemical methods

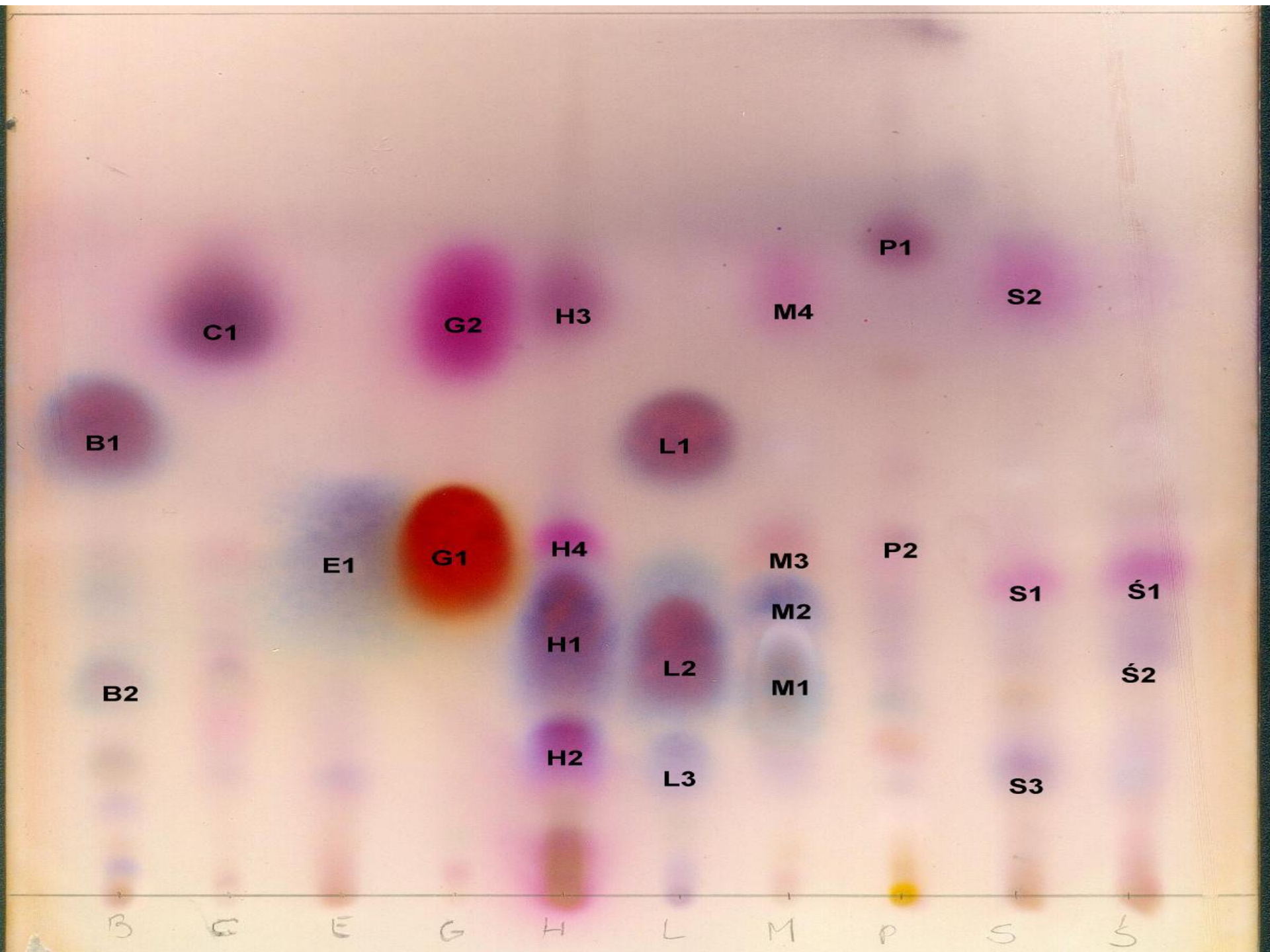
1 - Colorless or non fluorescent spots can be visualized by exposing the developed plate to iodine vapor . The iodine vapor interacts with the sample components to produce color .

2 - A common technique for organic compounds is spraying the plate with sulfuric acid solution and then heating it to char the compounds and develop black spots.

3 - Specific color reagents exist into which the TLC plate is dipped or which are sprayed onto the plate,

eg.2,4-dinitrophenylhydrazine for carbonyl compounds , ninhydrine for amino acids,




P1
S2
M4
H3
G2
C1
B1
L1
E1
G1
H4
M3
P2
S1
Ś1
M2
H1
L2
M1
Ś2
B2
H2
L3
S3
B
C
E
G
H
L
M
P
S
Ś

Applications

A- In Organic Chemistry

i-reactions are qualitatively monitored with TLC .

ii- separation of reaction products .

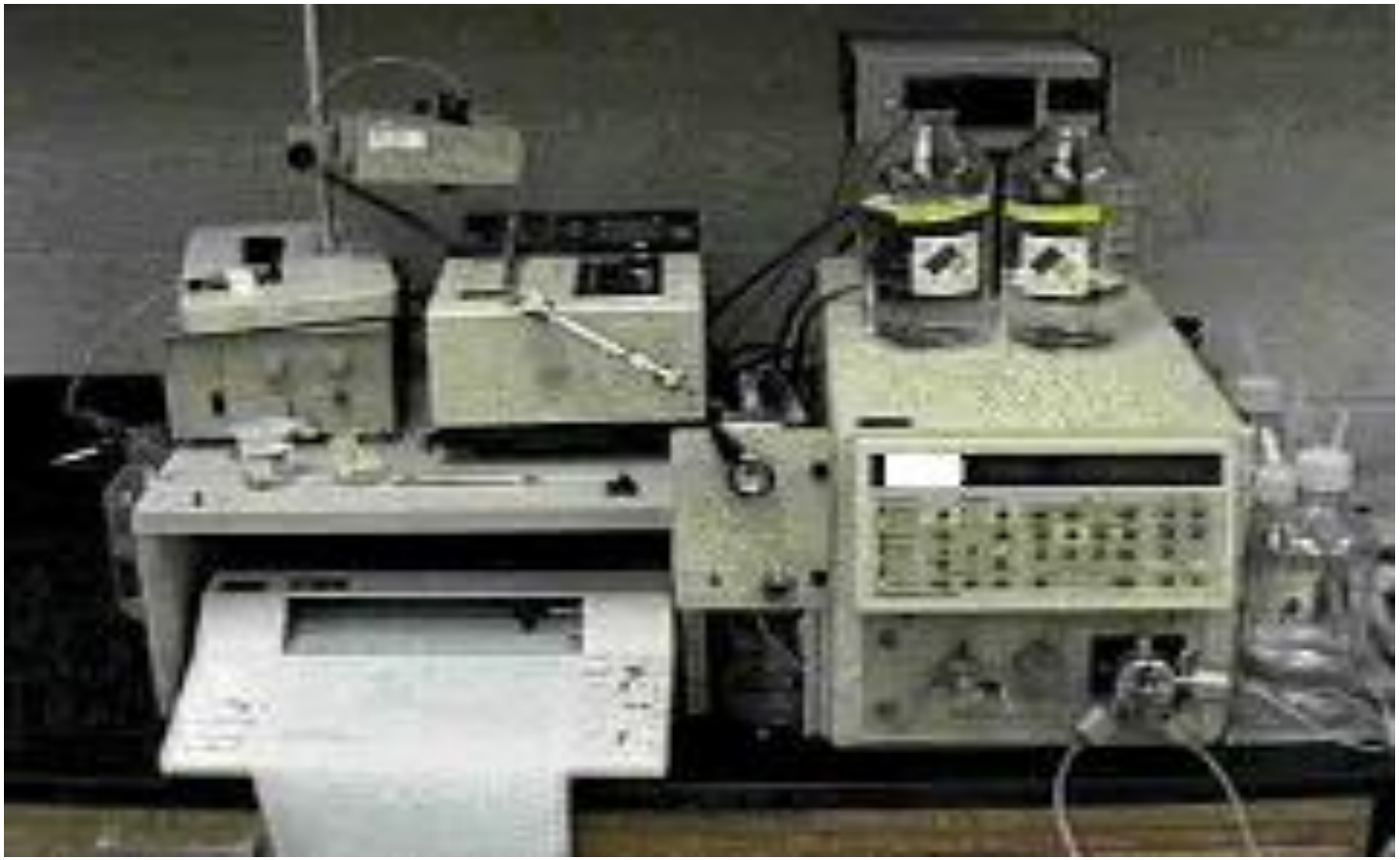
B – in Analytical Chemistry

i- qualitative tests by comparing RF values

ii- test for the purity of org. and pharmaceutical compounds

iii – quantitative analysis (by using scanner).

COLUMN CHROMATOGRAPHY
HPLC

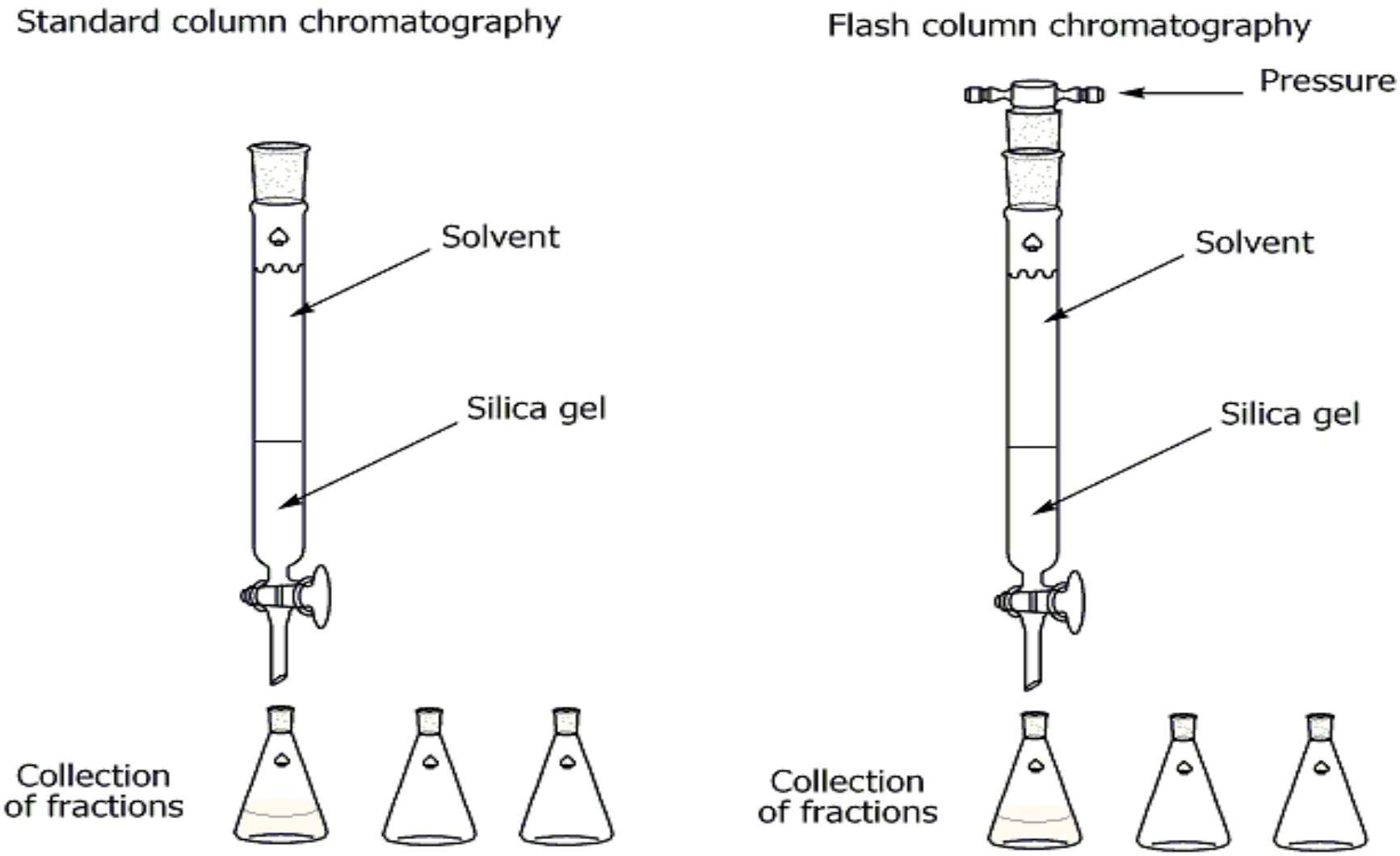


Theory

- Chromatography involves a sample (or sample extract) being dissolved in a *mobile phase* (which may be a gas, a liquid or a supercritical fluid). The mobile phase is then forced through an immobile, immiscible *stationary phase*. The phases are chosen such that components of the sample have differing solubilities in each phase. A component which is quite soluble in the stationary phase will take longer to travel through it than a component which is not very soluble in the stationary phase but very soluble in the mobile phase. As a result of these differences in mobilities, sample components will become separated from each other as they travel through the stationary phase.

- Techniques such as H.P.L.C. (High Performance Liquid Chromatography) and G.C. (Gas Chromatography) use *columns* - narrow tubes packed with stationary phase, through which the mobile phase is forced. The sample is transported through the column by continuous addition of mobile phase. This process is called *elution*. The average rate at which an analyte moves through the column is determined by the time it spends in the mobile phase.

diagram


Standard column chromatography
Solvent
Silica gel
Collection of fractions
Flash column chromatography
Pressure
Solvent
Silica gel
Collection of fractions

Column Efficiency

- **Column efficiency refers to the performance of the stationary phase to accomplish particular separations. This entails how well the column is packed and its kinetic performance.**
- **The time between sample injection and an analyte peak reaching a detector at the end of the column is termed the *retention time* (*t*R). Each analyte in a sample will have a different retention time. The time taken for the mobile phase to pass through the column is called *dead time* (*t*M).**

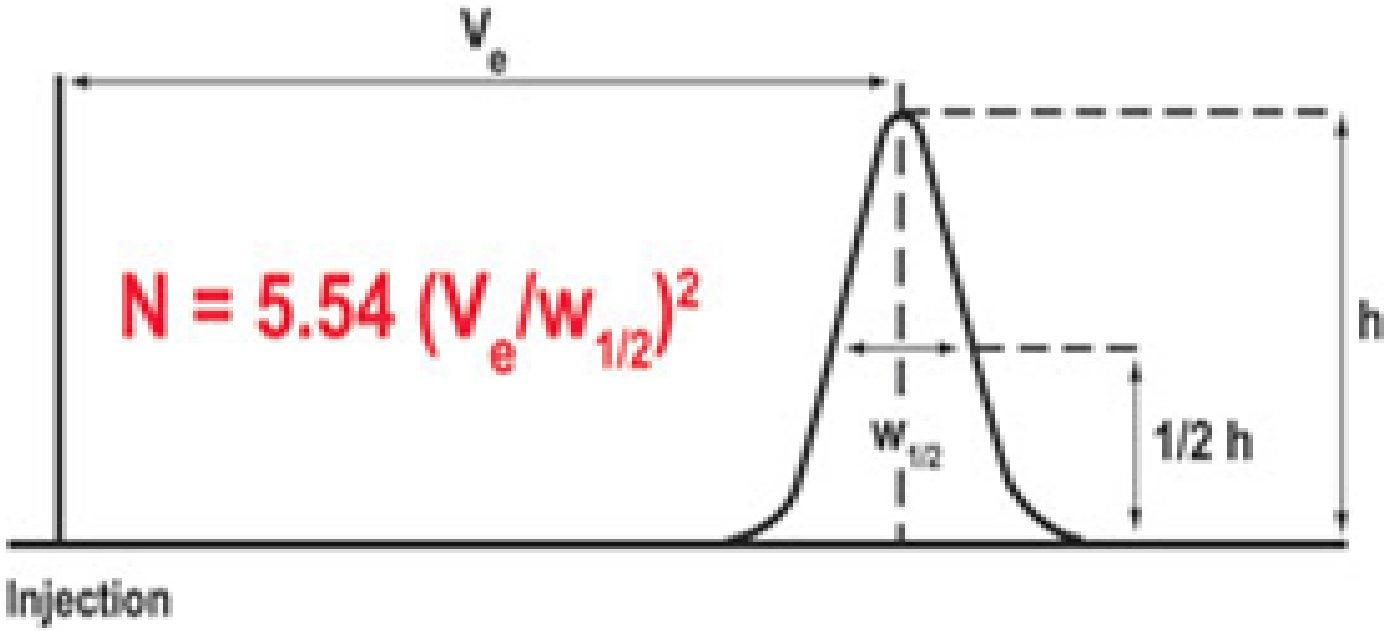
Column efficiency calculation

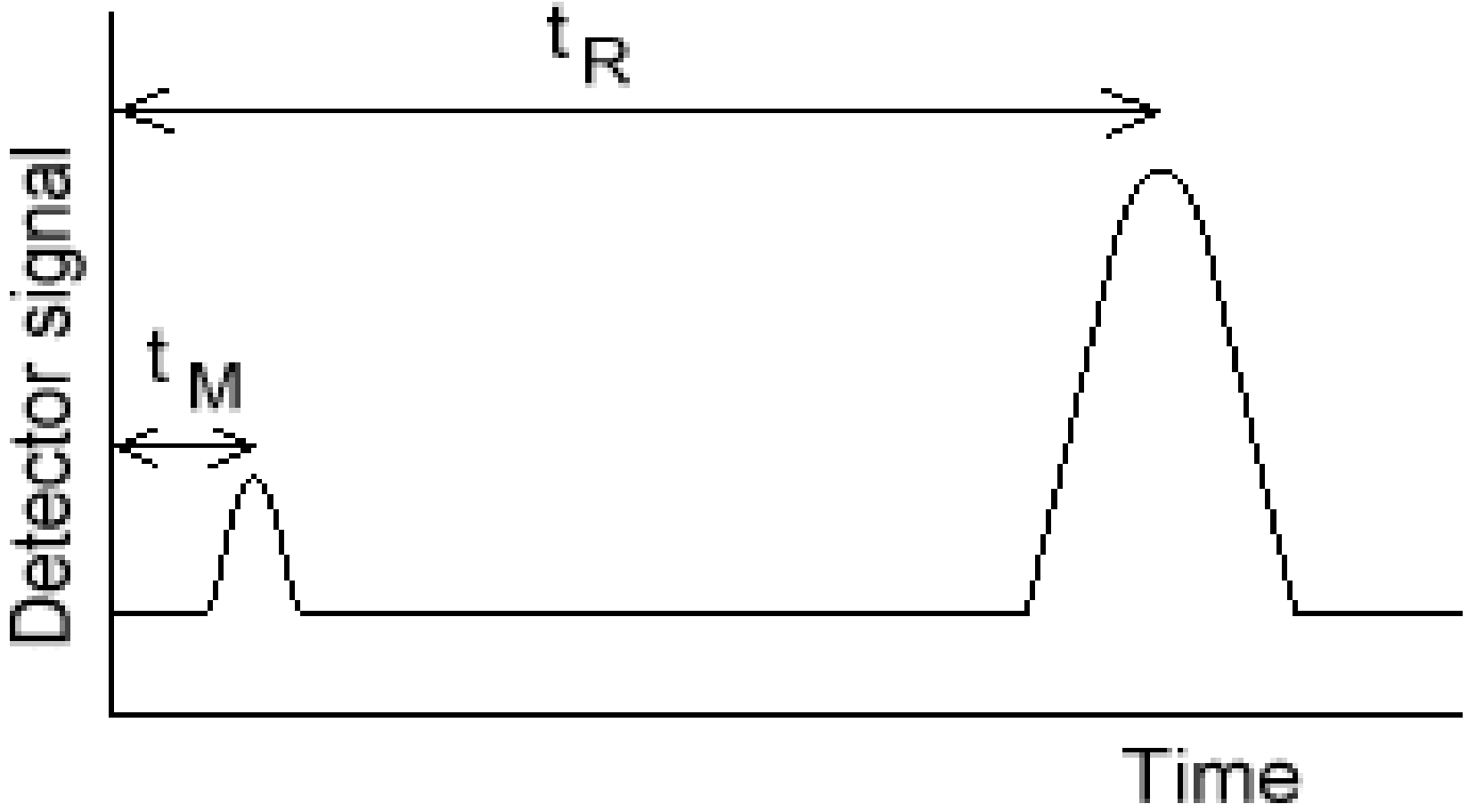
Column efficiency, indicated as the number of theoretical plates per column, is calculated as

$$N = 5.54 \, (t_R / w_{0.5})^2$$

where t_R is the retention time of the analyte of interest and

$w_{0.5}$ the width of the peak at half height.




t_R
t_M
Detector signal
Time

Calculation of column efficiency value:

All the following methods use this formula that measures N, or number of theoretical plates:

$$N = a \frac{t_r^2}{W^2}$$

a = constant dependent on height where peak width measured

t_r = retention time

W = peak width

- **A term called the *retention factor, k'*, is often used to describe the migration rate of an analyte on a column. You may also find it called the *capacity factor*. The retention factor for analyte *A* is defined as;**

$$k'_A = t_R - t_M / t_M$$

t_R and t_M are easily obtained from a chromatogram.

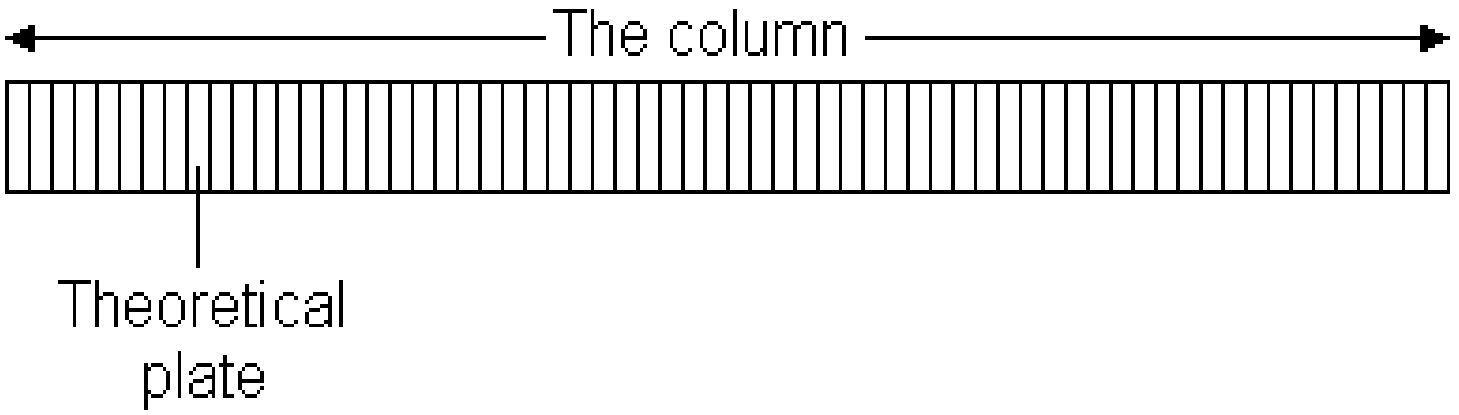
When an analytes retention factor is less than one, elution is so fast that accurate determination of the retention time is very difficult. High retention factors (greater than 20) mean that elution takes a very long time. Ideally, the retention factor for an analyte is between one and five.

- **We define a quantity called the *selectivity factor " ά"* , which describes the separation of two species (A and B) on the column;**

$$\alpha = k'_B / k'_A$$

- **When calculating the selectivity factor, species A elutes faster than species B. The selectivity factor is always greater than one.**

- **The Theoretical Plate Model of Chromatography**
- **The plate model supposes that the chromatographic column is contains a large number of separate layers, called *theoretical plates*. Separate equilibrations of the sample between the stationary and mobile phase occur in these "plates". The analyte moves down the column by transfer of equilibrated mobile phase from one plate to the next.**



- **It is important to remember that the plates do not really exist; They also serve as a way of measuring column efficiency, either by stating *the number of theoretical plates in a column, N* (the more plates the better), or by stating the plate height; the *Height Equivalent to a Theoretical Plate* (the smaller the better).**
- **If the length of the column is *L*, then the HETP is**

$$\text{HETP} = L / N$$

- **The number of theoretical plates that a real column possesses can be found by examining a chromatographic peak after elution;**

$$N = \frac{5.55\ t_R^{\,2}}{w_{1/2}^{\,2}}$$

- **where *w*1/2 is the peak width at half-height.**

- **Resolution**
- **Although the selectivity factor, "ά", describes the separation of band centeres, it does not take into account peak widths. Another measure of how well species have been separated is provided by measurement of the *resolution*. The resolution of two species, A and B, is defined as**

$$R = \frac{2[(t_R)_B - (t_R)_A]}{W_A + W_B}$$

- Baseline resolution is achieved when $R = 1.5$

- It is useful to relate the resolution to the number of plates in the column, the selectivity factor and the retention factors of the two solutes;

$$R = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{1 + k'_B}{k'_B}\right)$$

- One of the most serious problems involved in description of chromatographic peaks is tailing (forming of asymmetric peak) .
- Symmetry is calculated from the following formula : $T = \frac{W_{0.05}}{2f}$

where T is the tailing factor .

For a symmetric peak T must be near to 1 .

- To obtain high resolution, the three terms must be maximised. An increase in *N*, the number of theoretical plates, by lengthening the column, the height equivalent to a theoretical plate can be reduced by reducing the size of the stationary phase particles.
- It is often found that by controlling the capacity factor, *k'*, separations can be greatly improved. This can be achieved by changing the temperature (in Gas Chromatography) or the composition of the mobile phase (in Liquid Chromatography).

- The selectivity factor, "**ά**", can also be manipulated to improve separations. When "**ά**" is close to unity, optimising *k'* and increasing *N* is not sufficient to give good separation in a reasonable time. In these cases, *k'* is optimised first, and then "**ά**" is increased by one of the following procedures:
- Changing mobile phase composition
- Changing column temperature
- Changing composition of stationary phase
- Using special chemical effects (such as incorporating a species which complexes with one of the solutes into the stationary phase)

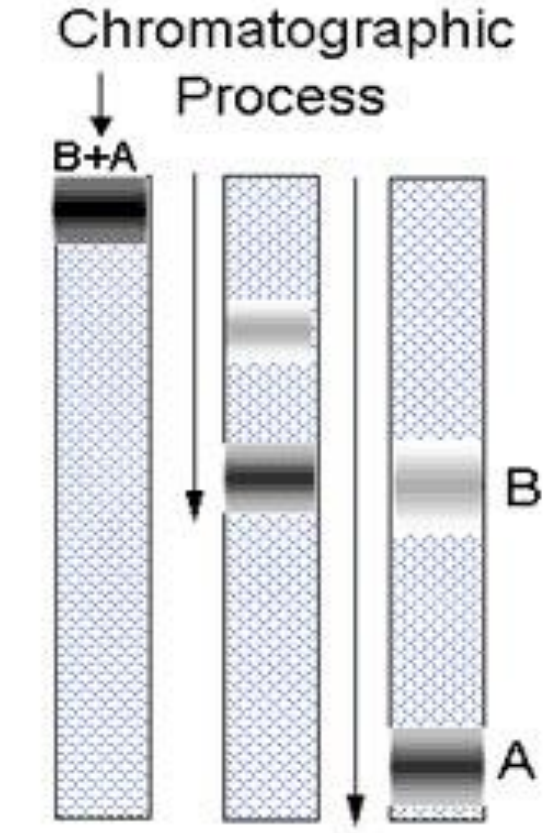
- **The separation of compounds can be described using several equations:**

EQUATIONS FOR ISOCRATIC ELUTION	
RETENTION TIME (s)	(1) $t_R = t_0 k' + t_0$
BANDWIDTH (ml)	(2) $\sigma = V_m(1 + k')N^{-1/2}$
RESOLUTION ($\Delta t_R/4\sigma$ or $\Delta t_g/4\sigma_g$)	(3) $R_s = [(1/4)(\alpha - 1)N^{1/2}][k'/(1 + k')]$
CAPACITY FACTOR	(4) $k' = (t_R - t_0)/t_0$

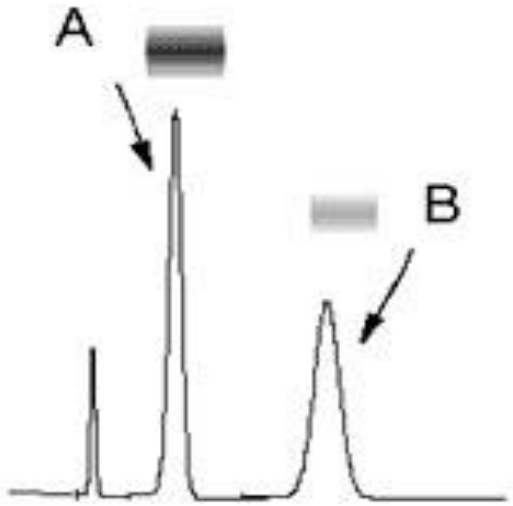
METHODS OF ELUTION

ELUTION

- **Introduction of the sample (dissolved in non polar solvent or the m.ph.) t1**
- **distribution of solutes**
  (a - adsorption b- extraction)
- **separation of solutes ;**
  difference in a- retention b- migration rate
- **isolation of the separated species by addition of sufficient fresh m.ph. (t2 , t3**
- **Detection ;**
  detector respond to solute concentration
- **chromatogram :**
  plot the detector signal As a function of time (used for both qualitative and quantitative analysis)



Elution through the Column



Chromatogram

A - Isocratic :

constant temperature (room temperature)

constant m.ph. composition with column temperature

constant interactive forces during elution

constant selectivity and retention

B - Flow programming

not used frequently in LC due to sensitivity of detector to change in flow rate.

- **Continues increase in flow rate excelerate elution of the last and more retained solutes .**

C - Temperature programming

used always in GC very effective , where tr α 1/T (i.e. tr reduced exponentially by increasing temperature .

D - Gradient elution :

In LC temperature programming is not effective as in GC because of the change in interactive forces in m.ph. and st.ph. , so tr is not so sensitive to change in temperature ,

- gradient means the composition of the m.ph. is continuously changed during development ; hence excelerate elution of the last and more retained solutes .

Types of HPLC

- **Normal phase HPLC**
- **this method separates analytes based on polarity;. NP-HPLC uses a polar stationary phase and a non-polar mobile phase, and works effectively for relatively polar analytes. The polar analyte associates with and is retained by the polar stationary phase. the interaction between the polar analyte and the polar stationary phase (relative to the mobile phase) increases the elution time. Use of more polar solvents in the mobile phase will decrease the retention time of the analytes while more hydrophobic solvents tend to increase retention times.**

- **NP-HPLC had fallen out of favor in the 1970's with the development of reversed-phase HPLC because of a lack of reproducibility of retention times as water or protic organic solvents changed the hydration state of the silica or alumina chromatographic media. Recently it has become useful again with the development of bonded phases which utilize a partition mechanism which provides reproducibility.**

Reversed phase chromatography

- Reversed phase HPLC (RP-HPLC or RPC) has a non-polar stationary phase and an aqueous, moderately polar mobile phase. One common stationary phase is a silica which has been treated with RMe_2SiCl, where R is a straight chain alkyl group such as $C_{18}H_{37}$ or C_8H_{17} With these stationary phases, retention time is longer for molecules which are more non-polar, while polar molecules elute more readily.
- An investigator can also increase retention time by adding a polar solvent to the mobile phase, or decrease retention time by adding a more hydrophobic solvent.

Size exclusion chromatography

- Size exclusion chromatography (SEC), also known as *gel permeation chromatography* or *gel filtration chromatography* ,separates particles on the basis of size.
- It is generally a low resolution chromatography and thus it is often reserved for the final, "polishing" step of a purification. It is also useful for determining the tertiary structure and quaternary structure of purified proteins.
- This technique is widely used for the molecular weight determination of polysaccharides.
- SEC is the official technique (suggested by European pharmacopeia) for the molecular weight comparison of different commercially available low-molecular weight heparins.

Ion exchange chromatography

- In Ion-exchange chromatography, retention is based on the attraction between solute ions and charged sites bound to the stationary phase.
- Some types of Ion Exchangers include:

  (1) Polystyrene resins- allows cross linkage which increases the stability of the chain. Higher cross linkage reduces swerving, which increases the equilibration time and ultimately improves selectivity.

  (2) Cellulose and dextran ion exchangers (gels)-These possess larger pore sizes and low charge densities making them suitable for protein separation.

  (3) Controlled-pore glass or porous silica.

- In general, ion exchangers favor the binding of ions of higher charge and smaller radius.
- An increase in counter ion (with respect to the functional groups in resins) concentration reduces the retention time.
- An increase in pH reduces the retention time in cation exchange while a decrease in pH reduces the retention time in anion exchange.
- This form of chromatography is widely used in the following applications:

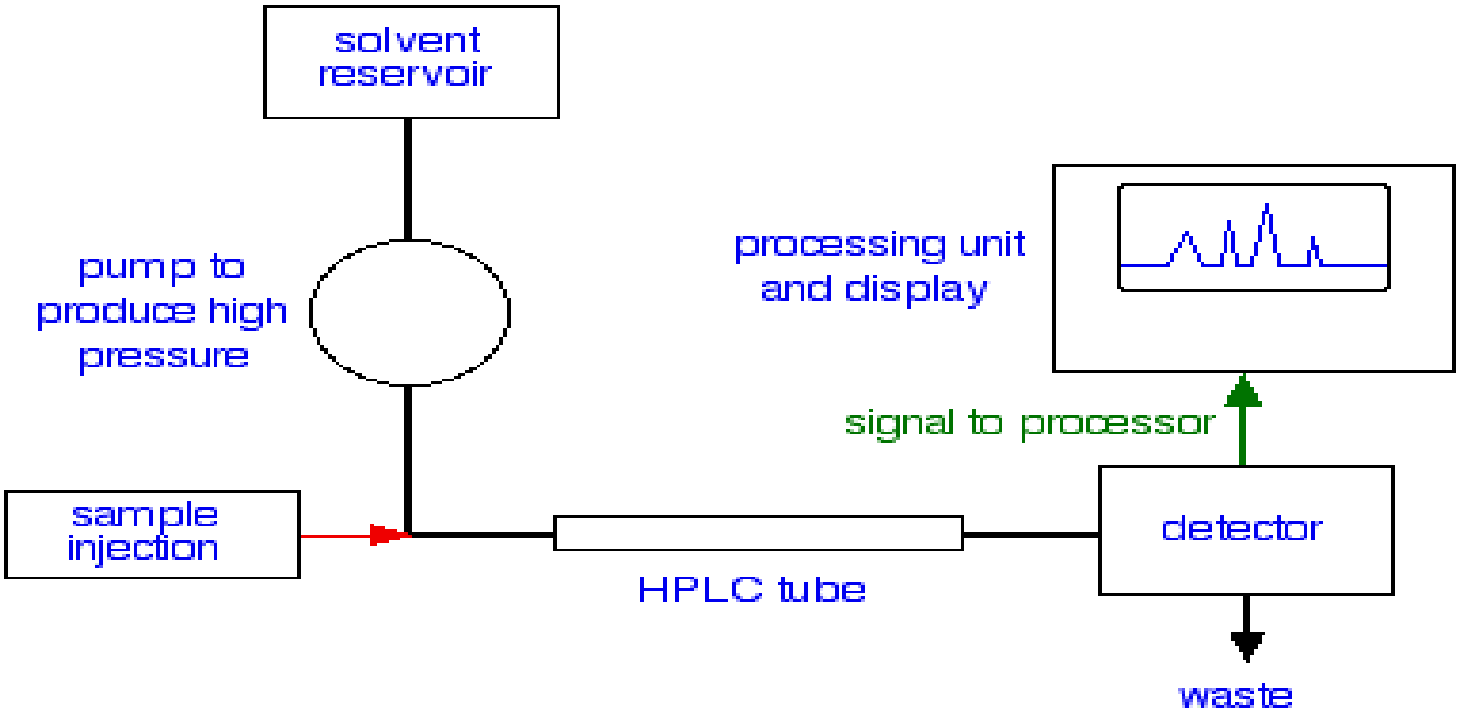
  In purifying water, preconcentration of trace components, Ligand-exchange chromatography, Ion-exchange chromatography of proteins, High-pH anion-exchange chromatography of carbohydrates and oligosaccharides, etc.

Instrumentation

- Scheme

Looking at the whole process

A flow scheme for HPLC



Mobile Phase

The mobile phase in HPLC refers to the solvent being continuously applied to the column, or stationary phase. The mobile phase acts as a carrier for the sample solution. A sample solution is injected into the mobile phase of an assay through the injector port. As a sample solution flows through a column with the mobile phase, the components of that solution migrate according to the non-covalent interactions of the compound with the column.

- **The chemical interactions of the mobile phase and sample, with the column, determine the degree of migration and separation of components contained in the sample.**
- **For example, those samples which have stronger interactions with the mobile phase than with the stationary phase will elute from the column faster, and thus have a shorter retention time, while the reverse is also true.**
- **The mobile phase can be altered in order to manipulate the interactions of the sample and the stationary phase. There are several types of mobile phases, these include: Isocratic, gradient .**

Injectors for HPLC

- **Samples are injected into the HPLC via an injection port. *The injection port* of an HPLC commonly consists of an injection valve and the sample loop. The sample is typically dissolved in the mobile phase before injection into the sample loop. The sample is then drawn into a syringe and injected into the loop via the injection valve. A rotation of the valve rotor closes the valve and opens the loop in order to inject the sample into the stream of the mobile phase. Loop volumes can range between 10 µl to over 500 µl. In modern HPLC systems, the sample injection is typically automated.**

HPLC Pumps

- **There are several types of pumps available for use with HPLC analysis, they are:**
- **Reciprocating Piston Pumps, Syringe Type Pumps, and Constant Pressure Pumps.**
- **Reciprocating Piston Pumps consist of a small motor driven piston which moves rapidly back and forth in a hydraulic chamber that may vary from 35-400 µL in volume. On the back stroke, the separation column valve is closed, and the piston pulls in solvent from the mobile phase reservoir. On the forward stroke, the pump pushes solvent out to the column from the reservoir. A wide range of flow rates can be attained by altering the piston stroke volume during each cycle, or by altering the stroke frequency. Dual and triple head pumps consist of identical piston-chamber units which operate at 180 or 120 degrees out of phase.**

Detectors

and Detection Limits

- **The detector for an HPLC is the component that emits a response due to the eluting sample compound and subsequently signals a peak on the chromatogram. It is positioned immediately posterior to the stationary phase in order to detect the compounds as they elute from the column. The bandwidth and height of the peaks may usually be adjusted using the coarse and fine tuning controls, and the detection and sensitivity parameters may also be controlled (in most cases).**

- **A detector operates by registering an output in response to sample detection. A linear relationship between response of the detector and concentration of the sample,**
- **There are many types of detectors that can be used with HPLC. Some of the more common detectors include:**
- **Refractive Index (RI), Ultra-Violet (UV), Fluorescent, Radiochemical, Electrochemical, Near-Infra Red (Near-IR), Mass Spectroscopy (MS), Nuclear Magnetic Resonance (NMR), and Light Scattering (LS).**

Columns

There are various columns that are secondary to the separating column or stationary phase. They are: Guard, Derivatizing, Capillary, Fast, and Preparatory Columns.

- **Guard Columns are placed anterior to the separating column. This serves as a protective factor that prolongs the life and usefulness of the separation column.**
- **They are dependable columns designed to filter or remove:**

1) particles that clog the separation column;

2) 2) compounds and ions that could ultimately cause "baseline drift", decreased resolution, decreased sensitivity, and create false peaks;

3) 3) compounds that may cause precipitation upon contact with the stationary or mobile phase; and

4) 4) compounds that might co-elute and cause extraneous peaks and interfere with detection and/or quantification.

These columns must be changed on a regular basis in order to optimize their protective function. Size of the packing varies with the type of protection needed.

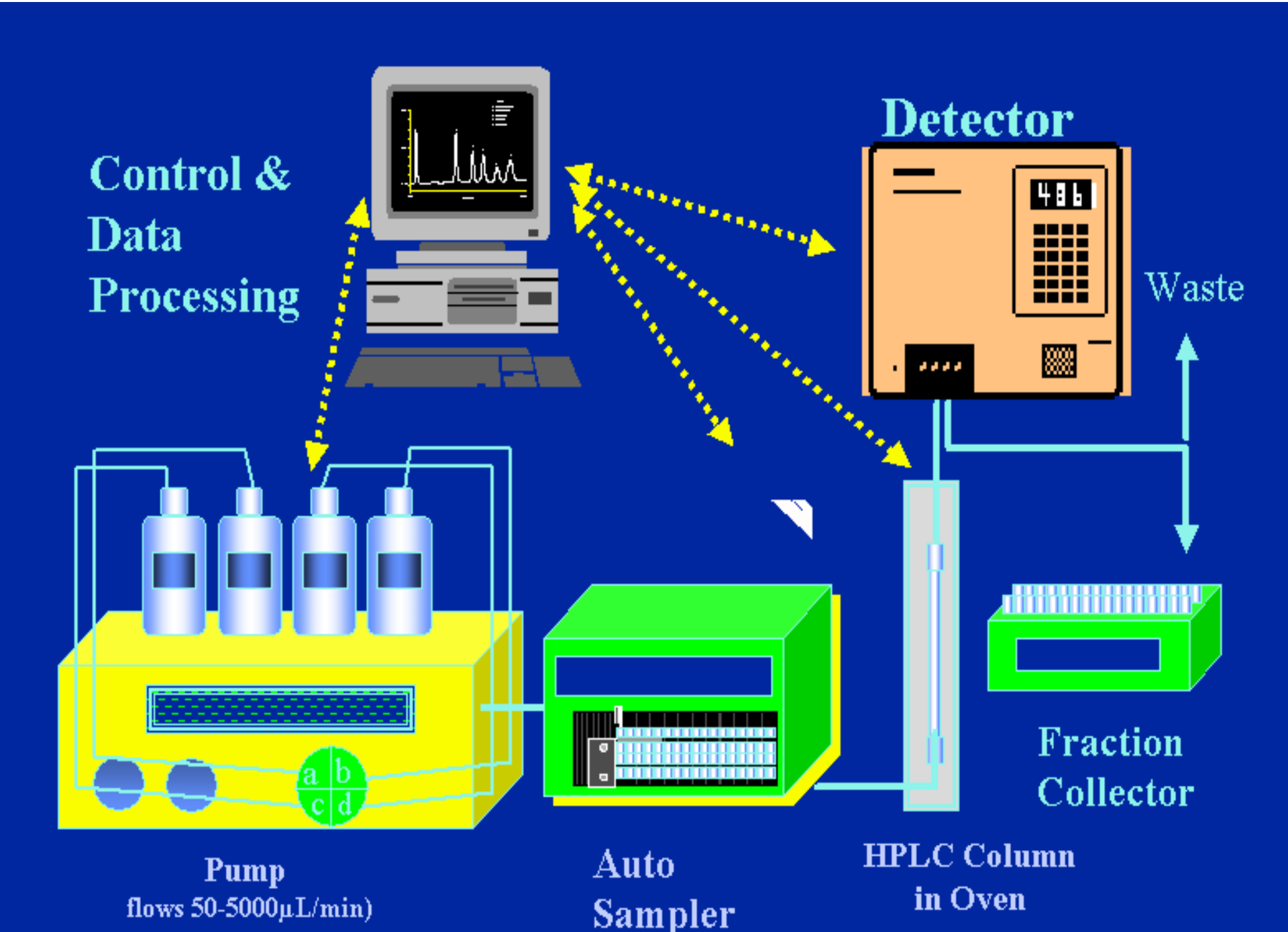
Applications for HPLC

- **HPLC has many applications including separation, identification, purification, and quantification of various compounds.**
- **Preparative HPLC refers to the process of isolation and purification of compounds. Important is the degree of solute purity and the throughput, which is the amount of compound produced per unit time.**
- **This differs from analytical HPLC, where the focus is to obtain information about the sample compound.**

- **The information that can be obtained includes identification, quantification, and resolution of a compound.**
- **Chemical Separations can be accomplished using HPLC by utilizing the fact that certain compounds have different migration rates given a particular column and mobile phase. Thus, the chromatographer can separate compounds (more on chiral separations) from each other using HPLC; the extent or degree of separation is mostly determined by the choice of stationary phase and mobile phase.**

- **Each compound should have a characteristic peak under certain chromatographic conditions.**
  Depending on what needs to be separated and how closely related the samples are, the chromatographer may choose the conditions, such as the proper mobile phase, to allow adequate separation in order to collect or extract the desired compound as it elutes from the stationary phase. The migration of the compounds and contaminants through the column need to differ enough so that the pure desired compound can be collected or extracted without incurring any other undesired compound.

- A -Qualitative analysis ; through comparison between t_R of standard and sample.
- B -Quantitative analysis.
- The area under the peak is proportional to the amount of X which has passed the detector, and this area can be calculated automatically by the computer linked to the display. The area it would measure is shown in green in the (very simplified) diagram.


Control &
Data
Processing
Detector
Waste
a b
c d
Fraction
Collector
Pump
flows 50-5000µL/min)
Auto
Sampler
HPLC Column
in Oven

Gas-liquid chromatography

- simply **gas chromatography** is a common type of chromatography used in organic chemistry for separating and analyzing compounds that can be vaporized without decomposition .
- **Typical uses** of GC include testing the purity of a particular substance, or separating the different components of a mixture .
- GC may help in identifying a compound.
- GC is very widely used for quantitative determination of many organic compounds.

- In gas chromatography, the *moving phase* (or "mobile phase") is a carrier gas , usually an inert gas such as helium or an unreactive gas such as nitrogen .The *stationary phase* is a microscopic layer of liquid or polymer on an inert solid support, inside a piece of glass or metal tubing called a column. The instrument used to perform gas chromatography is called a *gas chromatograph*

- The gaseous compounds being analyzed interact with the walls of the column, which is coated with different stationary phases. This causes each compound to elute at a different time, known as the *retention time* of the compound. The comparison of retention times is what gives GC its analytical usefulness.
- Gas chromatography is in principle similar to column chromatography (as well as other forms of chromatography, such as HPLC, TLC), but has several notable differences.
- Firstly, the process of separating the compounds in a mixture is carried out between a liquid stationary phase and a gas moving phase, whereas in column chromatography the stationary phase is a solid and the moving phase is a liquid.

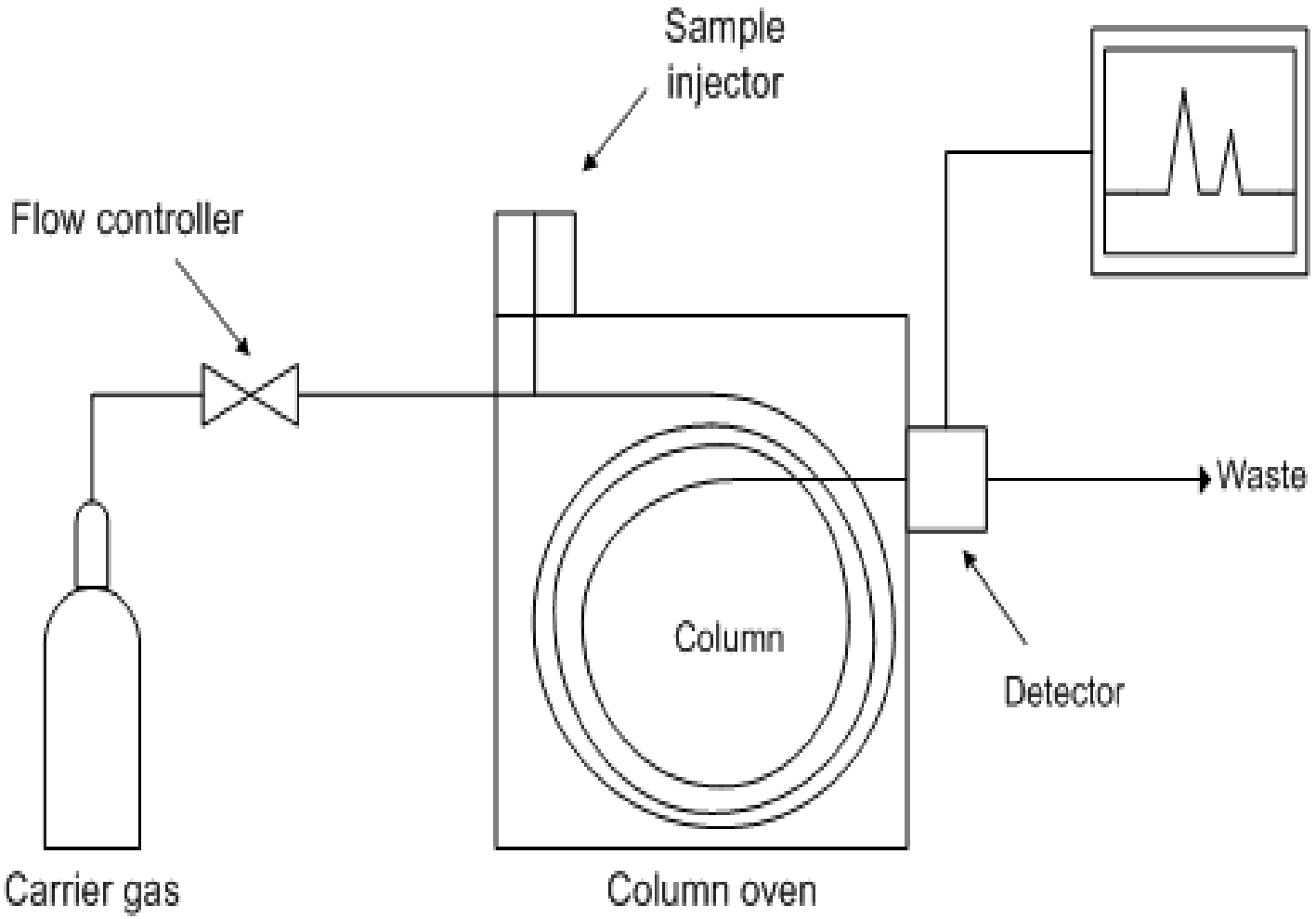
- Secondly, the column through which the gas phase passes is located in an oven where the temperature of the gas can be controlled,
- Thirdly, the concentration of a compound in the gas phase is solely a function of the vapor pressure of the gas .
- Gas chromatography is also similar to fractional distillation, since both processes separate the components of a mixture primarily based on boiling point (or vapor pressure) differences.

GC analysis

- A **gas chromatograph** is a chemical analysis instrument for separating chemicals in a complex sample.
- A gas chromatograph uses a flow-through narrow tube known as the *column*, through which different chemical constituents of a sample pass in a gas stream (carrier gas, *mobile phase*) at different rates depending on their various chemical and physical properties and their interaction with a specific column filling, called the *stationary phase*. As the chemicals exit the end of the column, they are detected and identified electronically. The function of the stationary phase in the column is to separate different components, causing each one to exit the column at a different time (*retention time*). Other parameters that can be used to alter the order or time of retention are the carrier gas flow rate, and the temperature.

- In a GC analysis, a known volume of gaseous or liquid analyte is injected into the "entrance" (head) of the column, usually using a microsyringe. As the carrier gas sweeps the analyte molecules through the column, this motion is inhibited by the adsorption of the analyte molecules either onto the column walls or onto packing materials in the column.
- The rate at which the molecules progress along the column depends on the strength of adsorption, which in turn depends on the type of molecule and on the stationary phase materials.
- Since each type of molecule has a different rate of progression, the various components of the analyte mixture are separated as they progress along the column and reach the end of the column at different times (retention time).

- A detector is used to monitor the outlet stream from the column; thus, the time at which each component reaches the outlet and the amount of that component can be determined. Generally, substances are identified (qualitatively) by the order in which they emerge (elute) from the column and by the retention time of the analyte in the column.


Sample
injector
Flow controller
Waste
Column
Detector
Carrier gas
Column oven

- **Inlets**
- The **column inlet** (or *injector*) provides the means to introduce a sample into a continuous flow of carrier gas. The inlet is a piece of hardware attached to the *column head*.
- **Columns**
- Two types of columns are used in GC:
- **Packed columns** are 1.5 - 10 m in length and have an internal diameter of 2 - 4 mm. The tubing is usually made of stainless steel or glass and contains a *packing* of finely divided, inert, solid support material (eg. diatomaceous earth) that is coated with a liquid or solid stationary phase. The nature of the coating material determines what type of materials will be most strongly adsorbed. Thus numerous columns are available that are designed to separate specific types of compounds.
- **Capillary columns** have a very small internal diameter, on the order of a few tenths of millimeters, and lengths between 25-60 meters are common. The inner column walls are coated with the active materials (WCOT columns), some columns are quasi solid filled with many parallel micropores (PLOT columns). Most capillary columns are made of fused-silica with a polyimide outer coating. These columns are flexible, so a very long column can be wound into a small coil.

- The **temperature-dependence** of molecular adsorption and of the rate of progression along the column necessitates a careful control of the column temperature to within a few tenths of a degree for precise work. Reducing the temperature produces the greatest level of separation, but can result in very long elution times. For some cases temperature is ramped either continuously or in steps to provide the desired separation. This is referred to as a **temperature program**. Electronic pressure control can also be used to modify flow rate during the analysis, aiding in faster run times while keeping acceptable levels of separation.
- The **choice of carrier gas** (*mobile phase*) is important, with hydrogen being the most efficient and providing the best separation. However, helium has a larger range of flow rates that are comparable to hydrogen in efficiency, with the added advantage that helium is non-flammable, and works with a greater number of detectors. Therefore, helium is the most common carrier gas used.

- **Detectors**
- A number of detectors are used in gas chromatography. The most common are the flame ionization detector (FID) and the thermal conductivity detector (TCD). Both are sensitive to a wide range of components, and both work over a wide range of concentrations. While TCDs are essentially universal and can be used to detect any component other than the carrier gas (as long as their thermal conductivities are different from that of the carrier gas, at detector temperature), FIDs are sensitive primarily to hydrocarbons, and are more sensitive to them than TCD. However, an FID cannot detect water. Both detectors are also quite robust. Since TCD is non-destructive, it can be operated in-series before an FID (destructive), thus providing complementary detection of the same analytes.

- Some gas chromatographs are connected to a mass spectrometer which acts as the detector. The combination is known as GC-MS .Some GC-MS are connected to an NMR spectrometer which acts as a back up detector. This combination is known as GC-MS-NMR

. Some are connected to an infrared spectrophotometer which acts as a back up detector. This combination is known as . GC-MS-IR

It must, however, be stressed this is very rare as most analyses needed can be concluded via purely GC-MS.

- **Methods**
- The **method** is the collection of conditions in which the GC operates for a given analysis.
- **Method development** is the process of determining what conditions are adequate and/or ideal for the analysis required.
- **Conditions** which can be varied to accommodate a required analysis include inlet temperature, detector temperature, column temperature and temperature program, carrier gas and carrier gas flow rates, the column's stationary phase, diameter and length, inlet type and flow rates, sample size and injection technique.

- **Carrier gas selection and flow rates**
- Typical carrier gases include helium ,nitrogen ,argon , hydrogen and air .Which gas to use is usually determined by the detector being used, for example, a FID requires helium as the carrier gas.
- Safety and availability can also influence carrier selection, for example, hydrogen is flammable, and high-purity . The purity of the carrier gas is also frequently determined by the detector.
- **Sample injection**

The real chromatographic analysis starts with the introduction of the sample onto the column.

Dissolved samples can be introduced directly onto the column via a COC injector.

gaseous samples (e.g., air cylinders) are usually injected using a gas switching valve system;

- Some general requirements, which a good injection technique should fulfill, are:
- It should be possible to obtain the column's optimum separation efficiency .
- It should allow accurate and reproducible injections of small amounts of representative samples .
- It should induce no change in sample composition. It should not exhibit discrimination based on differences in boiling point, polarity, concentration or thermal/catalytic stability .
- It should be applicable for trace analysis as well as for undiluted samples .

- **Column temperature and temperature program**
- The column(s) in a GC are contained in an oven, the temperature of which is precisely controlled electronically.
- The rate at which a sample passes through the column is directly proportional to the temperature of the column. The higher the column temperature, the faster the sample moves through the column. However, the faster a sample moves through the column, the less it interacts with the stationary phase, and the less the analytes are separated.
- In general, the column temperature is selected to compromise between the length of the analysis and the level of separation.

- A method which holds the column at the same temperature for the entire analysis is called "isothermal." Most methods, however, increase the column temperature during the analysis, the initial temperature, rate of temperature increase (the temperature "ramp") and final temperature is called the**" temperature program**".
- A temperature program allows analytes that elute early in the analysis to separate adequately, while shortening the time it takes for late-eluting analytes to pass through the column.

Application

- In general, substances that vaporize below ca. 300 °C (and therefore are stable up to that temperature) can be measured quantitatively. The samples are also required to be salt-free; they should not contain ions.
- for example in assuring the quality of products in the chemical industry; or measuring toxic substances in soil, air or water. GC is very accurate if used properly and can measure picomoles of a substance in a 1 ml liquid sample, or parts-per-billion concentrations in gaseous samples.
-