Column Chromatography

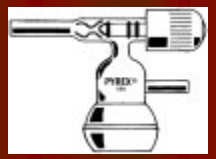
Types of columns:

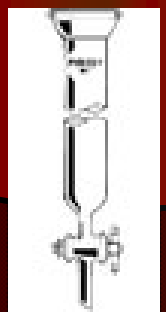
1- Gravity Columns:

The mobile phase move through the stationary phase by gravity force.

2- Flash Columns (Air or nitrogen pressure):

The mobile phase is pushed by stream of air or nitrogen using special using special values (Adaptors).





3-Low and Medium Pressure Columns (pumped):

The movement of mobile phase is accelerated by using pumps that generate low or medium pressure. The increase in the flow rate shorten the time of separation.

4-Vacuum Columns [Vacuum liquid chromatography (VLC)]:

The adsorbent is applied dry into a sintered glass funnel. The sample is applied by dry method or as solution. Then the mobile phase is added portion by portion and vacuum is applied after each portion to collect each fraction.

5- High pressure Columns (HPLC):

In this columns we use very fine silica gel so great increaser in separation power. However, the flow rate of the mobile phase is severely decreased. High pressure pumps are used to push the solvent through the column which in this case must be made of stainless steel.

Backing of Columns:

The adsorbent is applied to the Column in two ways:

- **Slurry packing (Wet method):**

  The adsorbent is suspended in the mobile phase and stirred very well to drive off all air bubbles. The resulted slurry is then poured into the column. At the tap end of the column a piece of glass wool or cotton must be added before the slurry application. Sand may be added after the slurry. After slurry application the column must be allowed to settle overnight.

- In gel chromatography the adsorbent must be soaked in the mobile phase overnight to absorb the mobile phase and swell.

- **2- Dry Packing:**

  In this method the dry adsorbent is poured to the column directly. Vibration is the applied to get rid of air bubbles then the mobile phase as passed through the adsorbent. This method can not be applied gel Chromatography.

- **Mobile phase:**

  It is a mixture of organic solvents (unusually one solvent only) the choice of the column mobile phase is achieved after TLC study in different solvent systems. Good solvent system must produce R_f value less than 0.6 for all materials to be separated by the column. If the system moves them more and produces higher R_f no separation will occur. Systems that do not move spots at all on TLC are not good for column separation.

- **Isocratic system:**

  Means using the same mobile phase from the beginning to the end of the separation.

- **Gradient:**

  The polarity of the system increased gradually during separation by increasing the proportion of the more polar solvent. A typical gradient may be start with $CHCl_3$, followed by $CHCl_3$/MeOH mixtures with gradual increase in % of MeOH till all spots are eluted from the system.

Monitoring the column:

- Usually fractions of certain volume are collected evaporated to small volume and spotted on TLC. Similar fractions are collected together for more purification or crystallization.
- In bioassay guided fraction the fractions are monitored by the bioassay then by TLC.

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Sample Application

- 1- Wet application: Dissolve the sample in the initial mobile phase and apply by pipette to the top of the column. This is very good method but in most of cases the samples are not soluble in the initial mobile phase.

- 2- Dry loading: Dissolve sample in any volatile solvent. The sample solution is then adsorbed on small weight of adsorbent and the solvent is allowed to evaporate. The dry adsorbent loaded with the sample is then applied to the column.

Theoretical cencopts

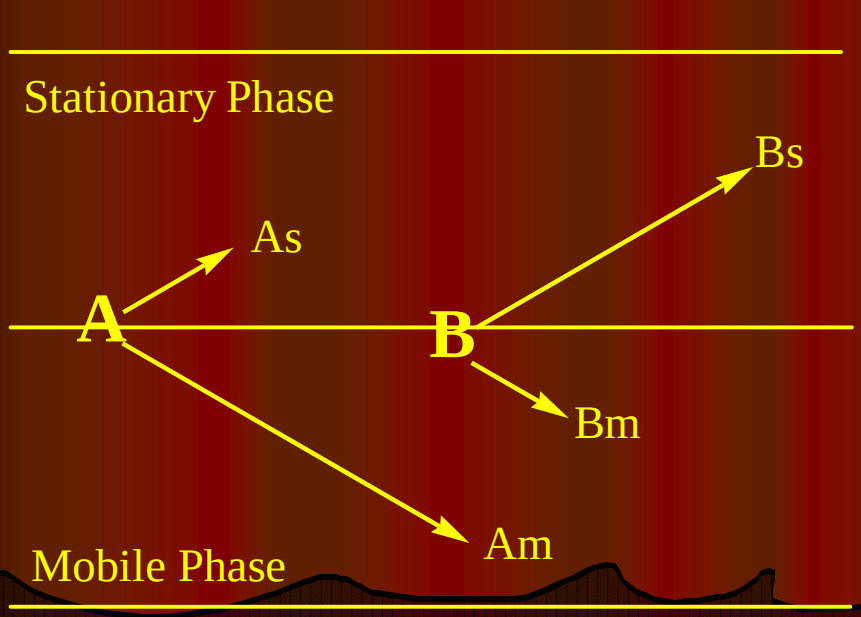
1- **Differential migration:**

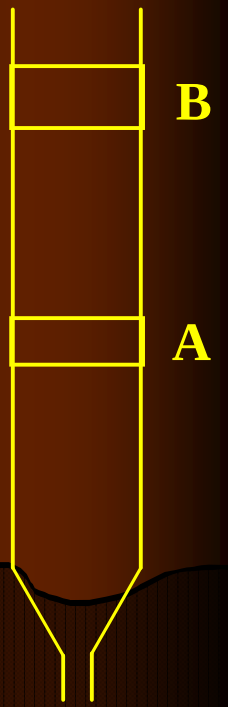
Different compounds move through the system with different rates of movement this is called "differential migration". The speed of any compound in the mixture is determined by the number of molecules of that compound in the mobile phase.

Suppose we have mixture of materials “A” and “B”:

A: Have more affinity to mobile phase ei large number of molecules are present in the mobile phase.

B: Have more affinity to stationary phase ei small number of molecules are present in the mobile phase.





Mathematical prsentation of differential migration:

- U: velocity of solvent (mobile phase).
- Ux: velocity of material X.
- R: fraction of material X in the mobile phase.

$$Ux = UR$$

- If R = I ie all the compound molecules are present in the mobile phase.

$$Ux = U \times I$$

$$Ux = u$$

:. Matenial X will move with the solvent velocity.

- if R = 0.0 ie all the compound molecules are present in the stationary phase.

$$Ux = u \times 0.0$$

$$\therefore Ux = 0.0$$

:.Material X will not move at all. For any material to be separated through the system it must be distributed between the mobile phase and stationary phase.

Capacity Factor:

- The factor that control the distribution of any material between the two competitive phases is called “Distribution coefficient” or “Capacity factor” or “Mass distribution ratio” K’

$$K' = \frac{(n)s}{(n)m}$$

(n)s : Is the total number of moles of the compound in the stationary phase.

(n)m : Is the total number of moles of the compound in the mobile phase.

$$K' = \frac{t_r - t_0}{t_0}$$

t_r : Time required for the sample to cross the column (retention time).

t_0 : Time required for the solvent molecules to cross the column.

- Bigger K’ means that the material retained more time on the column ei move slowly. Smaller K’ means faster movement.

2- Movement of materials through the chromatographic system in the form of “zones” or “bands”:

- It was assumed that the chromatographic system composed of number of “distribution systems” or “equilibrations” called “Theoretical Plates”. Each theoretical plat is composed of stationary phase and mobile phase. The height of each plate is called “Height equivalent to Theoretical Plate” (HETP).

- The number of theoretical plates “N” is important for separation. Increasing “N” resulted in narrower bands and better separation.

$$N = \frac{L}{HETP}$$

L: Column length

"N" can be increased by:

1- Increase the length of the column (impractical).

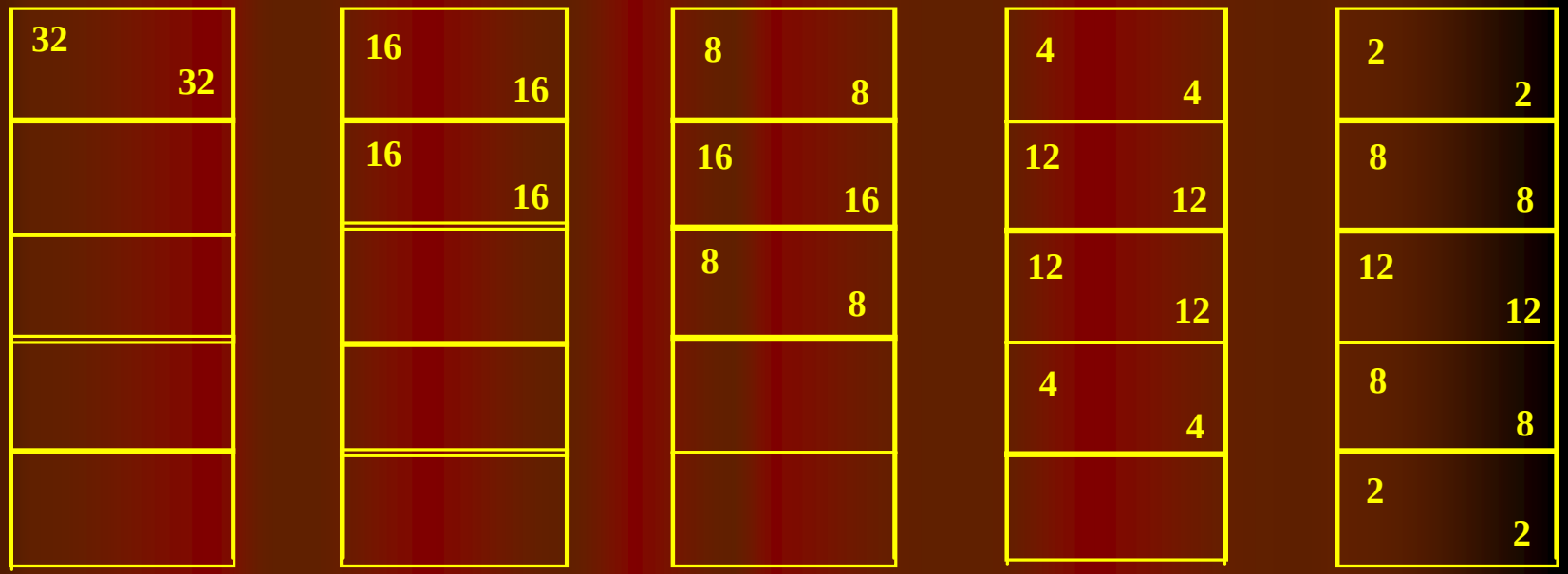
2- Decrease the HETP.

How to decrease HETP:

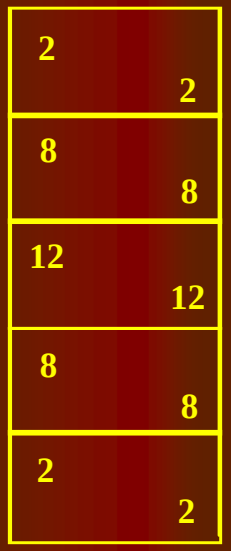
- Decrease the particle size of the stationary phase.
- Proper selection of good mobile phase.

Materials move through the column as bands or zones and velocity is controlled by K'.

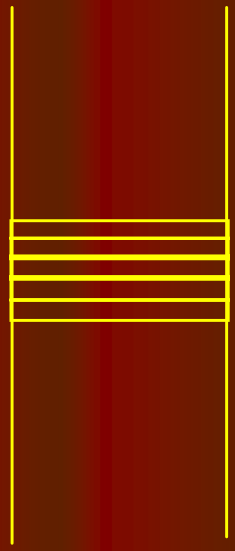
Material with K' = 1 (64 molecules)



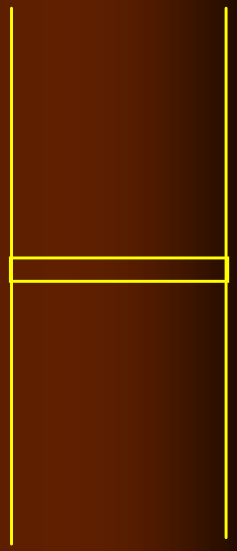
The material will be present in the middle of the system in the form of band. If we increase “N” the material will from narrower. Narrow bands allow better separation of mixtures.



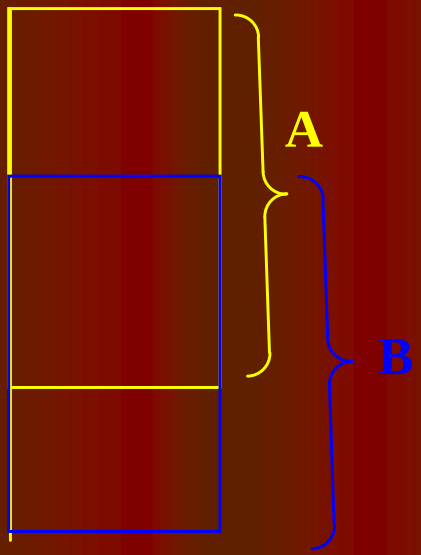
N= 5



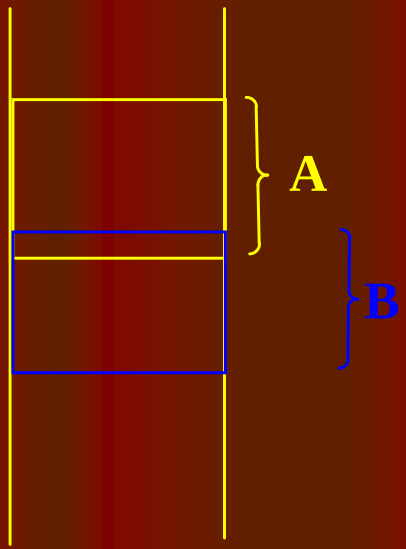
N= 25



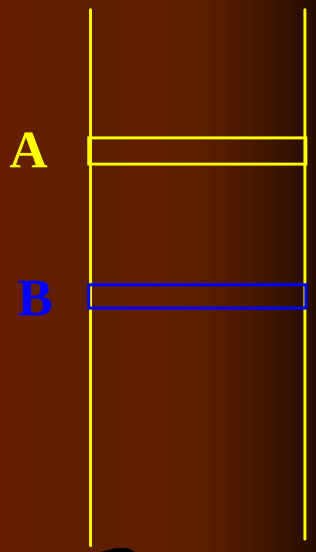
N= 150



N= 5



N= 25



N= 150

Factors affecting separation:

Factors due to Stationary Phase:

1- Particle size of the stationary phase: Reducing the particle size increases the surface area and improve separation. However, reduction of the particle size will decrease the flow rate of the mobile phase.

- In HPLC we use very fine particles to get very good separation. The flow rate problem is solved by the use high pressure pumps to push the mobile phase through the stationary phase. Columns are made of stainless steel to withstand the high pressure.

2- Adsorbent activity: The choice of the suitable adsorbent is very important.

3- Uniformity if packing of the column: If the stationary phase is not packed uniformly then the bands will be irregular and less uniform resulting in poor separation.

4- Concentration of the mixture: the proper ratio between sample to be separated and the amount of stationary phase is very important too much samples resulted in bad separation.

Factors due to Mobile Phase:

1- Selection of the proper mobile phase: Very polar mobile phase will wash out all components without any separation. On the other hand very non polar mobile phase will result in broad band and poor separation.

2- Rate of flow: Slower flow rate usually resulted in a better separation and narrower bands.

3- Consistency of flow: The continuous flow of the mobile phase during the whole experiment gives better separation than interrupting the flow then continue it later.

Factors due to Columns:

- Column dimensions: Increasing the length of the column improve separation. However, that usually leads to slower flow rate. Also increasing the column length some times is impractical.

- Column temperature: Increasing the temperature usually reduces the adsorption power of the stationary phase and increase elution speed. This may leads to decrease in the efficiency separation.