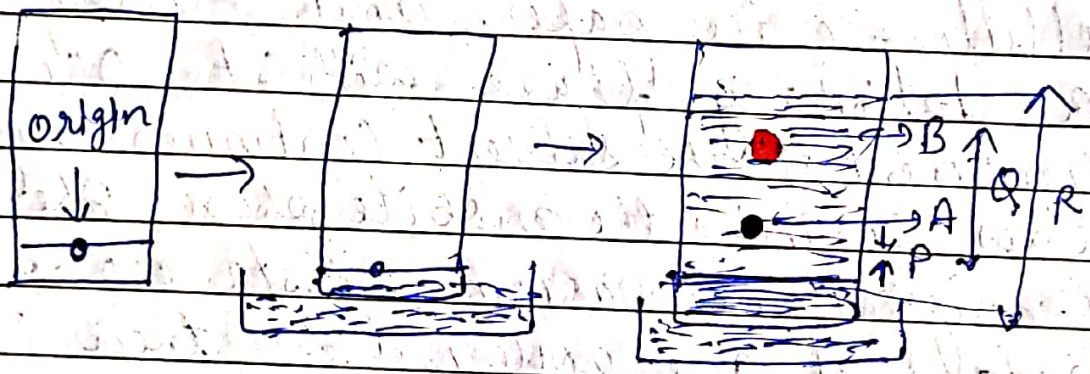
Paper & thin layer Chromatography

Paper chromatography is a type of partition chromatography. In paper chromatography, the end of the paper is dipped into solvent mixture consisting of aqueous and organic components. The solvent soaks into the paper by capillary action because of the fibrous nature of the paper. The aqous component of the solvent binds to the cellulose of the paper and thereby forms a stationary phase with it. The organic component of the solvent continues migrating, this forming the mobile phase. The rates of migration of various substances being separated are governed by their relative solubilities in the polar stationary phase and the non-polar mobile phase. During the separation process, a given solute is distributed between the mobile and stationary phases according to the partition co-efficient. The non polar molecules move faster than polar ones. The migration rate of substance during paper chromatography is usually expressed as the dimensionless term R_f (Relative front) which is the ratio of the distance travelled by substance and solvent front.

R_f → Distance travelled by substance / Distance travelled by solvent front

Naturally the R_f can be calculated only in those instances when the solvent is not allowed to leave the end of the paper sheet.



For A, $R_f = \frac{P}{R}$

For B, $R_f = \frac{Q}{R}$

Paper chromatography can be developed either by ascending or descending solvent flow. There is little difference in the quality of the chromatograms and the choice is usually a matter of personal preference. Descending chromatography has two ~~types of~~ advantages;

1. It is faster because gravity aids the flow

2. The quantitive separation of materials with very small Rf values, which therefore require long runs, the solvent can run off the paper.

~~Thin layer chromatography :-~~ [T.L.C]

Size exclusion Chromatography

Or Gel Chromatography

Size exclusion chromatography or molecular sieve chromatography separates molecules on the basis of size and shape. A column matrix is filled with porous gel beads, made up of an hydrated polymer such as polyacrylamide (Sephacryl or Biogel P) or dextran (Sephadex) or agarose (Sepharose) acts as a stationary phase. Size exclusion chromatography includes - Gel permeation chromatography & Gel filteration chromatography.

Gel permeation chromatography uses organic mobile solvent while gel filteration chromatography uses aq. mobile solvent to separate and characterize molecules.

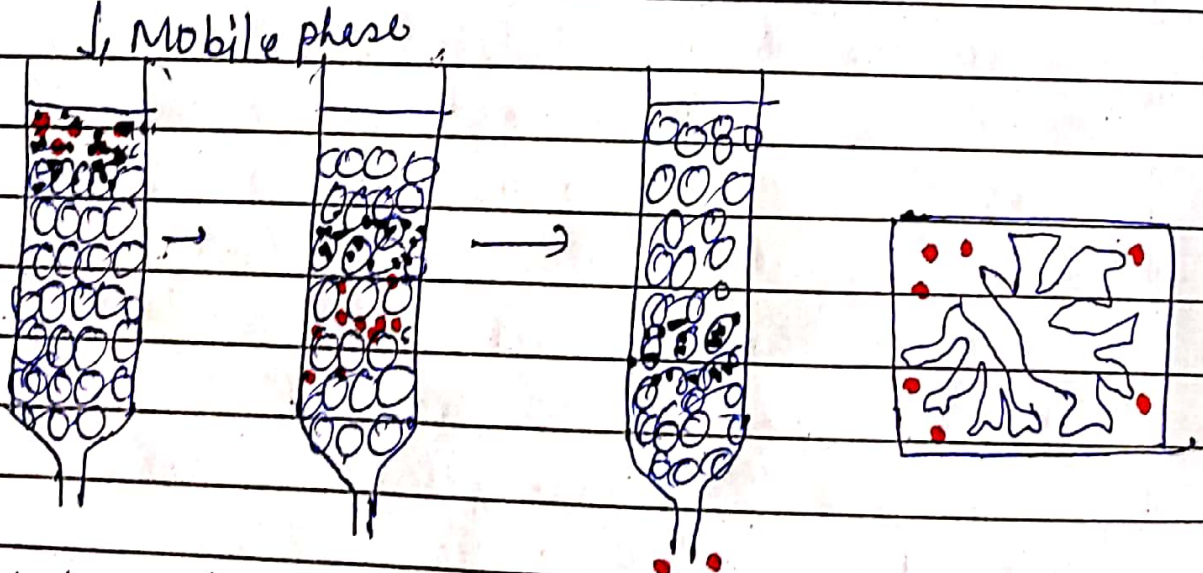


Fig - When the mobile phase passes through the porous gel beads small sample molecules which cannot enter the pores, pass through

the column at a faster rate than the small ones, correct pores sizes and solvents are crucial for a good separation.

The basis of size exclusion Chromatography is very simple. If a solution containing molecules of various sizes is passed through the column, molecules smaller than the pores can enter the pores in the beads whereas larger molecule cannot. So larger molecule moves faster and elute first. Since, smaller molecules can enter the pore present in the beads, they have longer path and longer retention time than larger molecules that cannot enter the pores.

Thus, if a mixture of proteins is applied to a column and then washed with an appropiate buffer, the first protein to emerge from the column are those that are too large to enter the pores of the gel beads. Other proteins are eluted in decreasing order of their molecular size.

The molecular mass of the smallest molecule unable to penetrate the pores of a given gel is said to be 'Gel's exclusion limit'. For ex- ex- The exclusion limit of a typical gel, Sephadex G-50 is 30,000 Da. All solute molecules

having a molecular size greater than this value would pass directly through the column bead without entering the gel pores.

For a given sample the distribution co-efficient K_d is dependent on size. Of the molecule is completely excluded by the pores, then $K_d = 0$; whereas if it enters into the porous beads and has accessibility to inner solvent, then $K_0 = 1$. For all other intermediate sizes, the Kd value will lie in the range of 0 - 1.

Size measurements by size exclusion chromatography:

In order to obtain size information about a solute from size exclusion chromatography experiment the column must be first be characterized in terms of the volumes accessible to analytes. The total volume (V_T) of a size exclusion chromatography column is devided into three parts;

1. The volume external to the packing material i.e void volum V_0;
2. The volume contained within the porous

beads that is accessible to small molecules, i.e internal volume V_i;

3. The volume occupied by the packing material itself i.e bed volume, V_g.

Therefore $V_T = V_0 + V_i + V_g$

The values of V_0 and V_i are determined experimentally by measuring the elution volumes of respectively, a large solute that is totally excluded from the interior of the porous bead and small solute that has access to all pores of the gel bead. The elution volume of a given solute V_e is the volume of solvent required to elute the solute from the column after it has first contacted the gel. The elution volume V_e of a solute that is partially included in the pores of the gel bead can be related to the void and internal volumes of column by the following equation:

$$V_e = V_0 + \sigma V_i$$ where, σ is the partition co-efficient of the solute.

It is the partition co-efficient σ which describes how much of the internal volume is available for the solute ($0 < \sigma < 1$) when σ is compared

with the values measured for solutes of known size, it provides information about the molecular size of an unknown solute. If a series of solutes of known size is subjected to size exclusion chromatography a linear relationship between partition co-efficient and size is observed.