Development of Chromatograms:

The development of a chromatogram is a crucial part of any of the chromatographic techniques. Clear separation of the employed for the development of a chromatogram. The advent of chromatographic development can be Arne Tiselius who categorized chromatographic development methods into three basic types which are:

(i) Frontal analysis

(ii) Displacement development

(iii) Elution analysis

(i) **Frontal analysis:** In the frontal analysis procedure of chromatographic development, the mixture to be separated is feed continuously into the column under suitable conditions that selectively favour the binding of all the components of the mixture excluding just one component. The component which is least retained in the stationary phase can be obtained in pure form at the column outlet. In this process of chromatographic development, no additional mobile phase is used. Fig. 6. describes the frontal analysis method of chromatographic development.

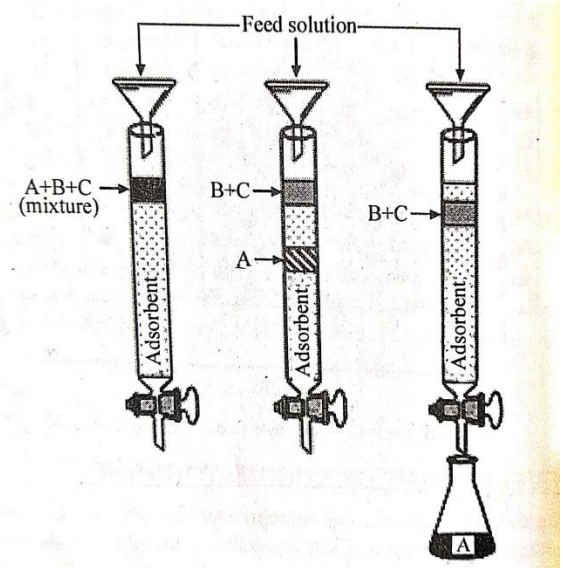

Fig. 6.1. Graphical representation of frontal analysis method.

This frontal analysis method is usually applied in the purification of biopolymers where the desired component to be separated has a much lower affinity for the stationary phase as compared to the rest of the feed component.

(ii) Displacement Development:

This chromatographic development procedure is employed as a preparative technique mainly in the pharmaceutical industry to purify or separate active ingredients of a complex mixture from impurities.

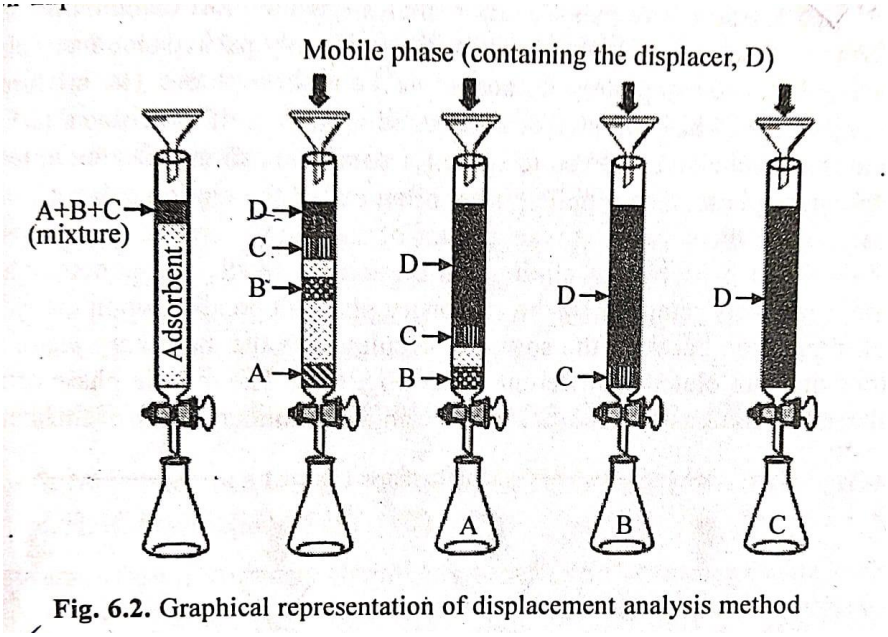

Fig. 6.2. Graphical representation of displacement analysis method

In this chromatographic development technique, a molecule called displacer is used to displace other analyte molecules that were already adsorbed into the stationary phase. In this development procedure, the mixture of sample to be separated is first loaded into the chromatographic bed containing the stationary phase. Then the mobile phase containing a suitable displacement reagent is allowed to pass through the chromatographic bed. The selection of the displacement reagent should be made in such a way that it has a very strong binding affinity towards the stationary phase as compared to all the individual components of the analyte. The displacer will displace the different components of the analytes at different retention times depending on their affinity of binding towards the stationary phase. The analyte component with least binding affinity will be displaced more easily than the others and will be eluted first. Thus, in the displacement chromatographic development technique, consecutive zones of separated components have been formed throughout the chromatographic bed and can be eluted in accordance with their relative affinity for the stationary phase. After displacing all

the analyte molecules, the chromatographic bed should be regenerated (i.e. complete removal of all the displacer molecules).

(iii) **Elution Analysis:** Elution analysis is the most common mode of chromatographic development where the uniformly packed stationary phase in a column is completely immersed in the mobile phase. The mixture of components to be separated is introduced at the top of the column to form a uniform zone and allowed the settle for some time so that it adheres to the stationary phase. The mobile phase, often called the eluting solvent is the poured into the column and the process of passing through the column is known as elution. As elution proceeds, component solutes are selectively retarded by the stationary phase depending upon the extent of interaction between the solute molecules with the stationary phase and thus they are eluted at different times (Fig. 6.3). The mobile phase carries the solute molecules down the column in a continuous series of transfers as the solute molecules partitioned themselves between the two phases.

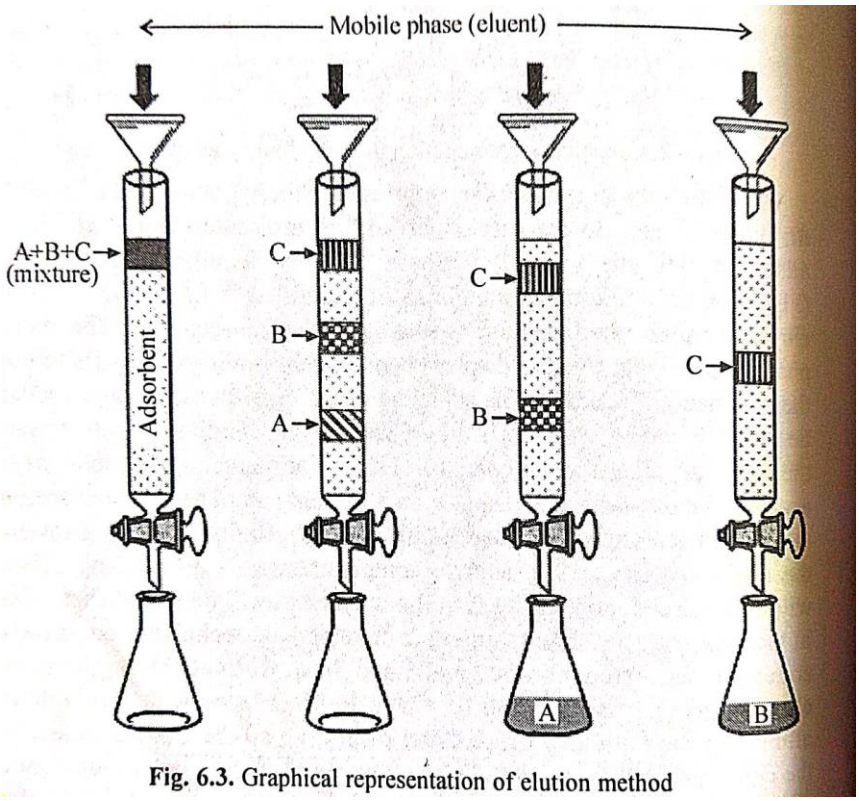

Fig. 6.3. Graphical representation of elution method

In other words, solutes having strong interaction with the stationary phase will be retained mostly in that phase and those having poor interaction with the stationary phase will spend mostly in the mobile phase. Thus, due to these differences, the rate of migration of different

components are different that causes them to separate into bands or zones along the length of the column. Pouring a sufficient quantity of the mobile phase through the column, the individual band of each component can be isolated.

Isocratic and Gradient elution :

Elution may be of two types isocratic and gradient. In the isocratic method, the mobile phase used is of the same composition during the separation process. But in the gradient method, the polarity or strength of the mobile phase is changed time to time to make analysis shorter or to get a better resolution of the components.