

### 3. Chromatography

What is it? Chromatography is an analytical technique for separating compounds on the basis of the differences in their affinity for a stationary phase and a mobile phase.

#### PRINCIPLES INVOLVED IN ADSORPTION CHROMATOGRAPHY

In adsorption, the binding of a compound to the surface of the solid phase takes place. Adsorption chromatography is a technique in which small differences in the adsorption behavior of substances between a moving solvent (liquid or gas) and a stationary solid phase are used to separate them. When the moving phase is a liquid it is called *liquid-solid chromatography* or *adsorption column chromatography*. When the moving phase is a gas it is called *gas-solid chromatography (GSC)*.

#### PARTITION CHROMATOGRAPHY

In partition, the relative solubility of a compound in two phases result in the separation of the compound. Partition chromatography is a technique in which mixtures of substances are separated by means of partition between the moving solvent and a stationary liquid, which is held on a suitable solid support. When the solvent (moving phase) is a liquid it is called *liquid-liquid chromatography*. When the solvent (moving phase) is a gas, the technique is called *vapour chromatography* or *gas-liquid chromatography*.

In the liquid-liquid chromatography, the solid support for the stationary liquid is provided by either cellulose or moist silica gel. This solid support may be in the form of this sheet. Such a technique is called *paper chromatography (PC)*. The solid support may be thin layers, then it is called *thin layer chromatography (TLC)*. The solid support may be a packed column, then it is called *partition column chromatography (PCC)*. The stationary liquid phase in all the above techniques is water.



## Ion exchange chromatography

Ion exchange is a process in which an interchange of ions like sign takes place between a solution and an insoluble solid (exchanger) in contact with the solution. This process is utilized to separate a mixture of ions. Here a reversible exchange of ions takes place between ions in a liquid phase (mobile phase) and an exchange resin (an insoluble substance containing ionic sites) which is the stationary phase.

## Paper chromatography

What is it? It is a chromatographic technique to analyse unknown substance and to separate mixtures of substances by means of solvents on specially designated filter paper.

**PRINCIPLES OF PAPER CHROMATOGRAPHY:** In paper chromatography (PC) the separation of unknown materials is affected by the differential migration of different components on a specially designed filter paper. This separation occurs due to the difference in partition coefficients.

It is a type of partition chromatography. Here the mobile phase is an organic solvent and the stationary phase is water absorbed on the surface of the paper. Alternatively the paper can be impregnated with anhydrous silica, alumina or ion exchange resin. Here partition occurs as a consequence of solid-liquid or liquid-liquid exchange equilibria.

**PAPER AS MEDIUM:** In paper chromatography, a coarse grade paper serves the role of a packed column in column chromatography. The role of a coated plate in TLC. In column chromatography and TLC silica gel acts as the solid support for the polar phase. In paper chromatography a filter serves this purpose. Thus paper serves as a medium for partition to take place between a stationary liquid and a mobile liquid.

**SOLVENTS USED:** As paper chromatography is based on partition of substances between two liquids, we have to use suitable solvents for stationary and mobile phases.

**Stationary phase:** The following classes of solvents are used as the stationary phase.

1. **Aqueous solvents:** Water is held firmly by paper. So it is used as the stationary phase. The filter paper is suspended in water placed in a closed chamber, whose atmosphere is saturated with water vapour and an equilibrium is allowed to be established.

2. **Hydrophilic solvents:** A hydrophilic organic solvent can be used as the stationary phase. Eg., methanol, formamide, glycol, glycerol etc.

3. **Hydrophobic solvents:** A hydrophobic solvents also can be used. E.g., Kerosene, hydrocarbons, dimethyl formamide etc.

**Mobile phase:** Several combinations are possible for the mobile phase. Mixtures of two, three or more solvents, solution of salts, buffers etc., are generally used. E.g., 1) Isopropyl alcohol, water and ammonia mixture in the ratio 9:2:1. 2) n-Butyl alcohol, water and acetic acid mixture in the ratio 4:5:1.

The choice of solvents depends on the nature of the substance to be separated. The choice is usually made by referring to the literature or by trial and error method.

**MECHANISM:** In this method, the dissolved substances are applied as a small spot on cellulose bound filter paper. The paper is then dipped into a vessel containing the mobile phase. The mixtures are partitioned between the solvent held on the paper (stationary phase) and organic solvent (mobile phase). The separation is effected by the differential migration of the mixture of substances.

Two types of forces operate when a drop of solution is applied on the filter paper and treated with a solvent.

i) The propelling force drags the substance in the direction of the flow of the solvent. The propelling force depends on the solvent power and the solubility of substances in the solvent. The compound with higher solubility will move rapidly along the paper than the less soluble one. This leads to a separation.

ii) The retarding force drags the substance behind towards its point of application. The retardation depends on the adsorption and partition. When a drop of the solute is treated with the solvent on the top of a paper, the more strongly adsorbed component will move along the paper with the solvent. The process of partition is also