Chromatography---

The collective term for a set of laboratory techniques for the separation of mixtures.

- Thin Layer Chromatography (TLC)
- Column Chromatography
- Gas Chromatography (GC)
- High Pressure Liquid Chromatography (HPLC)

Column Chromatography

(Please read Unit 9)

In column chromatography, the stationary phase, a solid adsorbent, is placed in a vertical glass (usually) column and the mobile phase, a liquid, is added to the top

and flows down through the column (by either gravity or external pressure).



Purpose

Column chromatography is generally used as a purification technique: it isolates a desired compound from a mixture. Column chromatography is separated into two categories, depending on how the solvent flows down the column. If the solvent is allowed to flow down the column by gravity, it is called **gravity column chromatography**. If the solvent is forced down the column by air pressure, it is called **flash column chromatography**, currently used in organic chemistry research laboratories.

The Solid Phase Adsorbent

Silica gel (SiO₂) and alumina (Al₂O₃) are two adsorbents commonly used by the organic chemist for column chromatography.

Interactions of the Compound and the Adsorbent

Compounds interact with the silica or alumina largely due to polar interactions. These interactions are discussed in the section on <u>TLC</u>. They are attractive interactions.

The Mobile Phase: Eluent Solvent

The process of washing a compound through a column using a solvent is known as *elution*. The solvent is sometimes known as the *eluent*.

The polarity of the solvent which is passed through the column affects the relative rates at which compounds move through the column. A highly polar solvent will move even highly polar molecules rapidly through the column. If a solvent is too polar, little or no separation of the components of a mixture will result. If a solvent is not polar enough, no compounds will elute from the column. Proper choice of an eluting solvent is thus crucial to the successful application of column chromatography as a separation technique. <u>TLC</u> is generally used to determine the system for a column chromatography separation.

Procedure

The mixture to be separated by column chromatography is applied to the top of the column. The solvent (the eluent) is passed through the column by gravity or by the application of air pressure. An equilibrium is established between the solute adsorbed on the adsorbent and the eluting solvent flowing down through the column. Because the different components in the mixture have different interactions with the stationary and mobile phases, they will be carried along with the mobile phase to varying degrees



and a separation will be achieved. The individual components, or eluents, are collected as the solvent drips from the bottom of the column.



Change the beaker once the yellow starts to drop through.

Analysis of Column Eluents

If the compounds separated in a column chromatography procedure are colored, the progress of the separation can simply be monitored visually. More commonly, the compounds to be isolated from column chromatography are colorless. In this case, small fractions of the eluent are collected sequentially in labeled tubes and the composition of each fraction is analyzed by thin layer chromatography (TLC).

The fractions that contain the desired compound are combined, and the solvent removed using a rotary evaporator. The purified compound is analyzed by IR and NMR spectroscopy.