SEPARATION AND ISOLATION OF CONSTITUENTS

As the instrumentation for the structure elucidation of organic compounds becomes ever more effective, and allows the use of increasingly small amounts of material, the most difficult operation in phytochemical research becomes that of the isolation and purification of plant constituents. Although the chemical properties of functional groups and moieties contained in compounds such as aldehydes, phenols and alkaloids can be exploited for their separation from other materials, such methods might not fractionate components of the same class; it is in this latter area that new techniques are constantly being developed.

Sublimation

Sublimation may sometimes be possible on the whole drug, as in the isolation of caffeine from tea or for the purification of materials present in a crude extract. Modern equipment employs low pressures with a strict control of temperature.

Distillation

Fractional distillation has been traditionally used for the separation of the components of volatile mixtures; in phytochemistry it has been widely used for the isolation of the components of volatile oils. On a laboratory scale it is not easy by this method to separate minor components of a mixture in a pure state and gas chromatography is now routinely used .

Steam distillation is much used to isolate volatile oils and hydrocyanic acid from plant material.

Fractional liberation

Some groups of compounds lend themselves to fractional liberation from a mixture. As an example, a mixture of alkaloid salts in aqueous solution, when treated with alkali, will give first the weakest base in the free state followed by base liberation in ascending order of basicity. If the mixture is shaken with an organic solvent after each addition, then a fractionated series of bases will be obtained. A similar scheme can be used for organic acids soluble in water-immiscible solvents; in this case, starting with a mixture of the acid salts, it is possible to fractionally liberate the acids by addition of mineral acids.

chromatography

- Some materials appear homogenous, but are actually a combination of substances. For example, green plants contain a mixture of different pigments. we can separate these materials by dissolving them in an appropriate liquid and allowing them to move through an absorbent matrix .Chromatography is a method used by scientists for separating organic and inorganic compounds so that they can be analyzed and studied . The word chromatography means "color writing" which is a way that a chemist can test liquid mixtures. While studying the coloring materials in plant life, a Russian botanist invented chromatography in 1903. the 'chromatographic procedure' originated by Tswett is one of the most useful techniques of general application. The use of charcoal for the decolorization and clarification of solutions is well known; coloured impurities . A light petroleum extract of green leaves is allowed to percolate slowly through a column of powdered calcium carbonate contained in a vertical glass tube. The pigmented contents of the solution are adsorbed on the substance of the column and undergo separation as percolation proceeds. The more strongly adsorbed pigments, xanthophyll and the chlorophylls, accumulate in distinct, characteristically coloured bands near the top of the column, while the less strongly adsorbed pigments, the carotenes, accumulate lower down.

- Chromatography is used in many different ways. It is used to determine what are unknown substances. The Police, and other detectives use chromatography when trying to solve a crime. It is also used to determine the presence of cocaine in urine, alcohol in blood and lead in water. Adsorption chromatography has proved particularly valuable in the isolation and purification of vitamins, hormones, many alkaloids, cardiac glycosides, anthraquinones , etc.

Partition chromatography

- Partition chromatography was introduced by Martin in 1941 for the separation of acetylated amino acids and was first applied to the separation of alkaloids by Evans in 1948. The separation of the components of a mixture is dependent on differences in the partition coefficients of the components between an aqueous and an immiscible organic liquid. The aqueous phase is usually the stationary phase and is intimately mixed with a suitable 'carrier' such as silica gel or paper In 1941 Martin introduced a method of partition chromatography using strips of filter paper as 'carriers' for the analysis of amino acid mixtures. The mixture of components to be separated is applied as a spot near one end of a prepared filter-paper strip. The paper is then supported in an airtight chamber which has an atmosphere saturated with solvent and water, and a supply of the water-saturated solvent. The most satisfactory solvents are those which are partially miscible with water, such as phenol, n-butanol and ethyl alcohol. Either the paper may be dipped in the solvent mixture so that the solvent front travels up the paper (ascending technique) or the trough of solvent may be supported at the top of the chamber, in which case the solvent travels down the paper (descending technique), Horizontal development and For the separation of some substances it is necessary to use a two dimensional chromatogram: first one solvent is run in one direction, then, after drying of the paper, a second solvent is run in a direction at right angles to the first—this is particularly applicable to mixtures of amino acids.

- After the filter-paper strips have been dried, the positions of the separated components can be revealed by the use of suitable developing agents: ninhydrin solution for amino acids; iodine solution or a modified Dragendorff's reagent for alkaloids; ferric chloride solution for phenols; alkali for anthraquinone derivatives . The ratio between the distance travelled on the paper by a component of the test solution and the distance travelled by the solvent is termed the R_f value and under standard conditions.

Adsorbtion chromatography

Thin-layer chromatography

- In 1958 Stahl demonstrated the wide applicability of TLC, a technique which had been known in principle for many years but was never developed. It has now achieved remarkable success in the separation of mixtures of all classes of natural products and is established as an analytical tool in modern pharmacopoeias Thin Layer Chromatography (TLC) is a solid-liquid technique in which the two phases are a solid (stationary phase) and a liquid (mobile phase). Solids most commonly used in chromatography are silica gel (SiO_2 x H_2O) and alumina (Al_2O_3 x H_2O). Both of these adsorbents are polar, but alumina is more so. Silica is also acidic. Alumina is available in neutral, basic, or acidic forms. Thin Layer Chromatography (TLC) is a sensitive, fast, simple and inexpensive analytical technique. It is a micro technique; as little as 10^{-9}g of material can be detected. Thin layer chromatography (TLC) is a widely employed laboratory technique and is similar to paper chromatography. However, instead of using a stationary phase of paper, it involves a stationary phase of a thin layer of adsorbent like silica gel , alumina, inert substrate . Compared to paper, it has the advantage of faster runs, better separations, and the choice between different adsorbents. For even better resolution and to allow for quantification.

- We have different types of silica gel depending on the number of free hydroxyl groups left on the silica gel:

- 1) Activated.

- 2) Inactivated.

  By the addition of water to silica gel we block the active sites of silica gel (deactivation) .if the silica gel have a large content of water, the water content is considered as a stationary phase and the mechanism of separation is partition.

- **TLC has advantages over PC**:

1. Fractionation and separation is more rapidly.

2. Better resolution where the adsorbent in TLC more compact.

3. More sensitive specially for small amounts.

4. Corrosive and drastic reagents used for the location of separated substances, as conc. sulfuric acid may destroy a paper chromatogram.

5. Certain additives may be added to the adsorbent to improve separation as boric acid, $AgNO_3$ and buffers.

6. Fluorescent inert may be incorporated to the adsorbent to facilitate detection especially in preparative TLC.

Types of adsorbents:

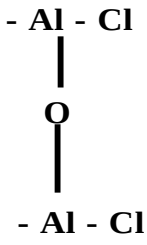
Alumina: alumina is a popular chromatographic adsorbent.

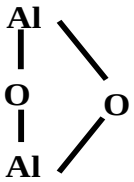
Commercial varieties of alumina available are:

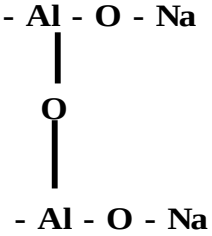
(a) Neutral alumina (pH 7-7.5), good for all purposes.

(b) Acidic alumina (pH 4): can be used as anion exchanger.

(c) basic alumina (pH 10): can be used as cation exchanger.







Acidic Alumina **Neutral almina** **Basic alunmina**

- **Silica** (**silica gel and silicic acid)**: Silica is one of the most widely used adsorbents in both column and thin-layer chromatography. The adsorption properties depend on the hydroxyl groups attached to silicon atoms. These hydroxyls interact with polar or unsaturated molecules by hydrogen bonding. Silica samples that have not been heated above 150°C, or exposed to water vapor after activation. This water blocks the underlying surface and renders the adsorbent less active. Heating silica between 100 and 120°C removes this water, without loss of surface hydroxyls, and gives adsorbent of maximum activity