**SEPERATION OF PLANT PIGMENTS BY COLOUMN CHROMATOGRAPHY**

**AIM**

To separate the plant pigments present in the given plant sample by column chromatography.

**INTRODUCTION**

Adsorption is a phenomenon in which compounds are held on the surfaces of a solid adsorbent, having specific adsorption sites, through weak non-ionic interaction such as Vander walls. Compounds bind with varying strength and hence can selectively absorbed. For good resolution selection of the right type of the absorbent and the eluent or mobile phase is essential. Some of the commonly used absorbents include charcoal, silica, alumina, hydroxyl apatite etc. eluent influences quality of separation since polarity of the mobile phase influences the absorption considerably. Non polar solvents favor maximum adsorption which decreases with increase in polarity of the solvent. Polar solvents are preferred for the substances having polar or hydrophilic groups. Non polar solvents for substances having hydrophobic and non - polar groups.

**Chemicals and other materials:**

Silica gel 60 [Merck], petroleum ether, acetone, NaCl, CaCo3, Na2Co3, fresh leaves.

**Apparatus and glass wares:**

Glass chromatography column with a porous membrane at the bottom and stopcock at the outlet, 5 measuring cylinder 25 ml, beaker 100 ml, beaker 600 ml, 9 Erlene Mayer’s flask, mortar and pestle, glass rod cork ring swan-neck lamp.

**PROCEDURE**

**Extraction of the leaf pigments:**

* Using a pestle, fresh leaves are grinded in a mortar containing 22ml of acetone, 3 ml of petroleum ether and a spatula tip full of CaCo3. The pigment extract is filtered.

**Eluting solvent [Mobile phase]**

Mixture of petroleum ether and acetone [7:3].

**Silica gel slurry:**

Using a beaker of an appropriate size, a slurry of silica gel and eluting solvent is prepared.

**Packing of the column:**

* A clean and dry column is aligned in a vertical position.
* A beaker is placed under the column outlet.
* The column is slowly and evenly filled about third full with silica gel slurry.
* The stop clock is allowed to open to allow liquid to drain in to the beaker.
* Pouring the slurry down a glass rod held against the wall of the column will minimize the bubbling and turbulence.
* The side of the chromatographic tube is gently trapped with a cork ring during the packing process to the silica gel compact.
* Meanwhile the stop clock is opened to allow the excess eluting solvent to run out.
* Using a powder funnel a small amount of sand is carefully added to the top of the silica gel column to prevent it being from disturbed when fresh solvent eluent is added.
* The solvent level is allowed to drop to 1 mm above sand.
* The bottom outlet of the separation column is closed. It is very important not to allow the column to run dry.

**EXPERIMENTAL PROCEDURE**

* Using a volumetric pipette 20 ml of the leaf extract is added directly [ or carefully down the side of the column] to the sand layer.
* Then the mobile phase is drained continuously to the top of the column by aid of a separation funnel.
* The bottom outlet of the column is opened.
* The effluent flows down through the column
* The column with the absorbent and the sample is developed. As the eluent passes down the column component of the mixture begin to move down the column.
* The separated zones flow out of the column where the elutes are collected in Erlenmeyer’s flask.
* The flask is changed as the elute change color.
* Using a swan-neck lamp a bright beam of light is directed at the leaf extract and at the samples eluted from chromatography column.

**RESULT**

The mobile phase slowly flows down through the silica gel column by gravity leaving behind zones of color the chromatography. The theory of column chromatography in analogous to that of thin layer chromatography. The different components mixture in the sample mixture pass through the column at different rates due to differences in their partitioning behavior between the mobile liquid phase and the stationary phase and thus the components are in dated.