

# **Separation of plant pigments by column chromatography**

**AIM-** The aim of the experiment is to separate plant pigments by using column chromatography.

## **BACKGROUND-**

The leaves of plants contain a number of colored pigments generally falling into two categories, chlorophylls and carotenoids. Chlorophylls a and b are the pigments that make plants look green. Carotenoids are part of a larger collection of plant-derived compounds called terpenes. Carotenoids are tetraterpenes (eight isoprene units). Lycopene, the compound responsible for the red coloring of tomatoes and watermelon, and  $\beta$ -carotene, the compound that causes carrots and apricots to be orange, are examples of carotenoids. Spinach leaves contain chlorophyll a and b and  $\beta$ -carotene as major pigments as well as smaller amounts of other pigments. Chlorophyll a and chlorophyll b are similar in structure and may not be able to be resolved in this procedure.

## **REQUIREMENTS-**

**Chemicals:** Petroleum ether (hexane) and acetone

**Apparatus:** Round bottom flask

Chromatography column

Pipettes

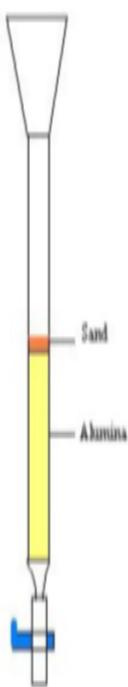
## **PROCEDURE-**

### **Extraction of the pigments**

About 5 grams of leaves is dried and placed in a mortar. Then the pigments are extracted by grinding the leaves with a pestle with about 5-10 ml of an 80:20 mixture (v/v), petroleum ether (hexane) and acetone. Then the liquid is decanted into a 50 ml round bottom flask. A quick filtration is done if necessary.

### **Preparation of the column**

Wet pack method is used to prepare the column. The chromatography column is made with plastic tip with frit, the one-way stopcock, and the plastic funnel. The column is filled with enough alumina to get the required height. The dry alumina is poured into a beaker and hexane (pet ether) is added. The mixture is swirled and then poured into the column. The column is tapped gently, so air is not trapped as the alumina settles. Then it is added with a small amount of sand after the alumina has been settled. The column should not contain air bubbles and should be homogeneous. Then the solvent level is allowed to drop to the level of the alumina sand intersection.



### **Running the column**

Using a long pipette, some of the pigment mixture is added directly onto the sand. Then it is added enough to fill the sand layer with color. Then the stopcock is opened and let the liquid level falls to the top of the alumina. Gently add petroleum ether to fill the sand layer. Then the stopcock is opened and let the liquid level fall to the top of the alumina. These steps are repeated at least three times or until all the colored compounds are in the alumina. Now the column is filled with petroleum ether.

*Do not ever let the column run dry. Never let the solvent level fall below the level of the alumina!*

Then the stopcock is opened to allow a drip rate of approximately 1 drop per second. First the yellow-orange  $\beta$ -carotene is eluted. As the yellow-orange colored product is eluted, it was collected in a test tube. 3. When the  $\beta$ -carotene has been eluted, the elutions of the chlorophylls are eluted by using a more polar solvent. Let the solvent level fall to the top of the alumina. Gently the column was filled with either pure acetone or a petroleum ether acetone combination. By using 70% petroleum ether: 30 % acetone combination, it might be able to separate chlorophylls a and b. Then the chlorophylls are collected in a separate test tube.

### **CONCLUSION-**

The R values of the above plant pigments are \_\_\_\_\_.

### **REFERENCES-**

1. [www.web.fscj.edu/milczanowski/ten/chloro.pdf](http://www.web.fscj.edu/milczanowski/ten/chloro.pdf).

