

# Separation Of Leaves Pigment In Case Of “Endod” Leaves By Using Thin Layer Chromatography (TLC)

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**Abstract:** Chromatography is one of the analytical methods that able to separate components of the plant leaves sample based on their interaction between stationary phase and mobile phase. Thin layer chromatography has valuable role to separate pigments in leaves based on the rate of reaction with mobile phase. The ratio of RF values are 0.73, 0.93, 0.55, and 0.35 for blue green, golden, yellow and yellow green colour respectively. The ratio RF values occurred between 0 and 1, the largest ratio the better resolution, while the smallest ratio the poor resolution.

**Keywords:** Chromatography, Thin layer chromatography, Retention factor, Mobile phase, Stationary phase.

## 1. Introduction

Chromatography was invented by the Russian botanist Mikhail Tswett shortly after the turn of century. He employed the technique to separate various plant pigments, such as chlorophyll and xanthophylls, by passing solution of them through glass columns packed with finally divide  $\text{CaCO}_3$  [calcium carbonate]. The separated species appeared as colored band on the column. Which accounts for the name he choose for the method [chromatography] which mean Greek chrome meaning <<colour>> and graphein meaning” to write [1]. In 1906 he reported separating different colored constituents of leaves by passing an extract of the leaves through a column of  $\text{CaCO}_3$  alumina and sucrose. He coined the term chromatography. Tswett's original experiments went virtually a noticed in the literature for decades, but eventually other method where developed and today there are several different types of chromatography. Chromatography is taken now to refer generally to the separation of components in a sample by distribution of the components between two phases, one stationary phase and other mobile phase [2].

The international union of pure and applied chemistry (IUPAC) has drafted recommended definition of chromatography as chromatography is the physical method of separation in which the components to be separated are distributed between two phase (stationary and mobile phase) in definite directions [3]. In june 1941 the British chemist A.J.P martin and R.L msynge presented a paper at Bio chemical society meeting in London on the separation of mono amino carboxylic acids in wool using a new liquid-liquid chromatography technique called partition chromatography. For this work they received the 1952 nobel prize in chemistry in 2<sup>nd</sup> paper they stated <<the mobile phase need not be liquid but may be a vapor.>> and very refined separation of volatile substance should therefore be possible in columns in which permanent gas is made to flow over gel impregnated with a non volatile solvent .>> but this was largely missed during world war II. When many libraries did not receive journals and it was not until 1950 that martin, along with young Colleague A.T. James, success fully demonstrated <<liquid gas partition chromatography. At the October meeting of the Biochemical society Thusthey bore two of the most powerful analysis techniques in the today. For a fascinating historical account of these developments

[2].Chromatography is one of the analysis methods that able to separate components of the fresh plant leave sample based on their interaction between stationary phase and mobile phase. Thin layer chromatography has valuable role to separate pigments in leaves based on the rate reaction with mobile phase. Therefore it becomes my concern area to conduct project on the separation of pigments of plants since plants are the living nature that sustain and determine the rest life kind and there is no project or research that has been investigated. Moreover, to check efficiency of thinlayer chromatography(TLC).

## 2. Materials and chemicals

### 2.1. Materials

Thin layer chromatography (TCL) marked at 2cm from one end

- Ruler, - Heat mantle
- Pencil - Condenser
- Capillary tube, - Sox-hlet
- extractor
- Chamber cover ( watch glass), - Round bottom flask
- Electro balance, - Metal stand
- Cylinder
- Pestle
- Mortar
- Beaker

### 2.1.1. Chemicals and reagents

- Diethyl ether
- Acetone
- Water
- Ethanol

### 2.2. Methodologies

#### 2.2.1. Sampling

Fresh “Endod” leave was collected and brought to the laboratory and dried then cut into pieces in order to decrease

surface area. Then 10g of the sample was taken for sample preparation.

### 2.2.2. Sample preparation

The dried leaves were grinded using mortar and pestle. By using soxhlet extractor the green color was extracted from leaves. The total amount of extracted was 6ml.



Figure 1:-Soxhlet extraction

### 2.2.3. Sample analysis

A small spot was applied on the marked point of the plate using capillary tube and dried to avoid touching the surface of the paper. Oils in skin villa solvent front alter adsorption properties. After it is dried the plate was suspended in chamber in such a way that not to touch the surface of the chamber. Using rubber, the tip of the plate was contacted with the solvent and covered with watch glass. The solvent phase and sample spot did not contact each other to prevent disturbance in separation and stood for 30 minutes. The pigments were gradually separated into individual components of different elution rate. Each component was measured and recorded. The retention factor (RF) for each component was calculated by the formula.

$$RF = \frac{\text{Distance moved by solute}}{\text{Distance moved by solvent}}$$

## 3. Results and discussion

As the solvent rise by capillary action up through the TLC plate the components of the pigments mixture are partitioned between the mobile phase (solvent) and the stationary phase (silicagel) due to their different adsorption and solubility strength. The more strongly a given component is adsorbed to the stationary phase, the less easily it is removed by mobile phase. The more weakly components are adsorbed the faster it migrates up TLC plate, on the other hand the running distance depends on the solubility of the pigment in the solvents. The pigments (components) were separated at different elution rate depending up on the interaction with the stationary phase and mobile phase. The component that reacted actively with mobile phase at fast rate than the component reacts less actively. Then the individual component is visible; therefore each component moved at a certain fraction of the rate along with the solvent and their RF values were calculated. The visible components are blue green = 3.3 cm, Golden = 4.2 yellow = 2.5 cm yellow green = 1.6cm and the distance of solvent = 4.5 cm.

$$RF = \frac{\text{Distance of solute}}{\text{Distance of solvent}}$$

$$RF \text{ blue green} = \frac{\text{distance of blue green}}{\text{distance of solvent}} = \frac{3.3 \text{ cm}}{4.5 \text{ cm}} = 0.73$$

$$RF \text{ golden} = \frac{\text{distance of golden}}{\text{distance of solvent}} = \frac{4.2 \text{ cm}}{4.5 \text{ cm}} = 0.93$$

$$RF \text{ yellow} = \frac{\text{distance of yellow}}{\text{distance of solvent}} = \frac{2.5 \text{ cm}}{4.5 \text{ cm}} = 0.55$$

$$RF \text{ yellow green} = \frac{\text{distance of yellow green}}{\text{distance of solvent}} = \frac{1.6 \text{ cm}}{4.5 \text{ cm}} = 0.35$$

**Table 4.1:** shows the Rf – value obtained when the two solvent are applied to separate of each component of pigment and comparison with literature review.

No	color formed	Distance solute (cm)	Distance. solvent (cm)	Rf	from literature review	
					Type of pigments	Rf
1	golden	4.2	4.5	0.93	carotene	0.95
2	blue green	3.3	4.5	0.73	chlorophyll 'a'	0.60
3	yellow	2.5	4.5	0.55	lutein	0.50
4	yellow green	1.6	4.5	0.35	chlorophyll 'b'	0.35

- Golden color was observed because of the presence of carotene pigment in "Endod" leaf. The RF value was calculated to be 0.93 which is similar with the work that has been conducted earlier. One of the technique to identify the type of pigment is using reference or known materials. Therefore the work compared with the reference whose Rf value = 0.95 I, E high resolution
- Blue green color was observed because of the presence of chlorophyll 'a' pigment in "Endod" leaf that RF – value was obtained to be 0.73 which nearly related with the work that has been done before. This method to determine the type of pigment known materials which its Rf value = 0.60
- Yellow color was observed because of the presence of lutein pigment in "Endod" leaf that Rf – value was determined to be 0.55 which almost the same as the work that has been done, its Rf – value 0.50.
- Finally yellow green color was observed because of the presence of chlorophyll 'b' pigment in "Endod" leaf that RF – value was calculated to be 0.35 that exactly the same as the work that conducted earlier that its Rf – value 0.35 i.e. poor resolutions.
- Generally the comparison between my experiment in case of "Endod" leaf and the one that has been already done in case of other leaves are similar. Even though all are not exactly the same since it produced from different leaves.

## 4. Conclusion and recommendation

### 4.1. Conclusions

The application of developing solvent is to carry up the component based on their interaction with mobile phase and stationary phase. The mechanisms of separation each component was gradually separated into four (4) different components. And the ratio of  $R_f$  – values occurred between 0 and 1, the largest the ratio the better resolution, while the smallest the ratio the poor resolution. Generally, thin layer chromatography is the most powerful for separating technique.

### 4.2. Recommendation

- ✓ When compare my result and that obtained from literature review it almost similar properties.
- ✓ The retention factor ( $R_f$ ) – value of each component of “Endod” is nearly similar to the  $R_f$  – value that had already done .
- ✓ The distance moved by solvent is two third of the total length of thin layer chromatography (9.33 cm). But from my experiment the total length of the solvent moved was 4.5 cm. therefore this not two – third of the total length. Those due to lack of some chemicals and perfect instrument.
- ✓ This project increases the interest of student and made awareness technique and determines how to improve the application of thin layer chromatography.
- ✓ The  $R_f$  – values of separated component depend on the number of factors, including the rate of migration of each component in a sample mixture. For this reason that standards used should be as nearly like the actual sample as possible.

## 5. References

- [1] Douglas A. Skoog, Donald M. West an introduction to chromatographic method, pp 660, standford university.
- [2] Gary D, Christian, (1971), chromatography principles and theory, pp-555, Russia .
- [3] L.S. Ettre, (1993), “Nomenclature for chromatography” pure and applied chemistry, pp 1819- 72, Russia.
- [4] B, Fried and J. sherma, (1999), thin layer chromatography 4<sup>th</sup>ed, pp65 – 70, New York.