International Conference on "Latest Innovations in Engineering, Management, Social Science and Education"
The International Centre Goa, Panjim, Goa (India)
28th October 2018 www.conferenceworld.in ISBN:978-93-87793-54-5

PLANT PIGMENT PAPER CHROMATOGRAPHY

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ABSTRACT:

This experiment is to separate plant pigments and to calculate r_f value of the pigments separated from plant

1. INTRODUCTION

Paper chromatography is a technique used to separate substances in a mixture based on the movement of thedifferent substances up a piece of paper by capillary action. Pigments extracted from plant cells contain a variety of molecules, such as chlorophylls, beta carotene, and xanthophyll, that can be separated using paper chromatography. A small sample of plant pigment placed on chromatography paper travels up the paper due to

capillary action. Beta carotene is carried the furthest because it is highly soluble in the solvent and because it forms no hydrogen bonds with the chromatography paper fibers. Xanthophyll contains oxygen and does not travel quite as far with the solvent because it is less soluble than beta carotene and forms some hydrogen bonds with the paper. Chlorophylls are bound more tightly to the paper than the other two, so they travel the shortest distance.

1.1 PLANT PIGMENTS

For Photosynthesis plants use light energy, which is converted to chemical energy. To absorb the light energy to power the chemical reactions. Plant pigments are macromolecules produced by plants. This pigments the specified wavelength of visible light. The reflected wavelength are the colors we see in plant while observing(eg:- green pigments reflect green light)

Plant contain different pigments with different color and some which are observed are..

- Chlorophylls(green)
- Carotenoids(yellow, orange red)
- Anthocyannis (red to blue, depending on pH)
- Betalains (red or yellow)

1.2 CHROMATOGRAPHY

Chromatography separates molecules because of different solubilities of molecules in selected sample

The distance travelled by pigment is unique for every pigment in set conditions. The ratio is Retention Factor (R_f)

$$Rf = \frac{\text{distance solvent travels (cm)}}{\text{distance pigment travels (cm)}}$$

2. EQUIPMENT AND MATERIAL

- 2 or 3 fresh spinach leaves
- Ruler
- Large Test tube
- Cork with push pin
- Chromatography solvent(precut 18 cm strips)

- Chromatography solvent(9:1 petroleum ether or acetone)
- 6 ml syringe
- Colored pencils
- Calculator
- Scissors
- Plastic wrap
- 70% isopropyl alcohol
- Plastic pipettes
- 5 test tubes (20-30 mL)
- Test tube Rack
- Sharpie markers or tape (for labelling test tubes)
- 4-6 spectrophotometer cuvettes
- Test tube rack for cuvettes
- Kimwipes
- Genesis 20 Spectrophotometer

3. SAFETY MEASURES

Wear goggles and aprons when working with chemicals.

Petroleum ether, acetone and alcohol are volatile and flammable.

Avoid breathing vapors of reagent it is poisonous

4. PROCEDURE

4.1 DAY 1

4.1.1 PREPARATION OF SAMPLE

1. Take a strip of chromatography paper approx. 18 cm long which has its one end as blunt and other as pointed.

2. With the help of pencil draw a line 2 cm from pointed end

3. Attach a cork with push pin by bending the blunt end of the paper. Adjust length of paper so that it barely touches the end of test tube without any bending

4. Place a ruler over the leaf so that the ruler covers the line made by pencil from pointed end.

5. With the help of coin press the leaf so that it forms a definite green line

6. Allow the line to dry

7. Repeat the process till the pencil line is completely covered by the narrow green band

4.1.2 SEPARATION OF PIGMENTS

1. Use a 6 ml syringe to dispense 5 ml of chromatography solvent in test tube.

2. Carefully lower the strip in the test tube and secure the cork in top. The solvent should touch the pointed end but not the green line

3. Be careful not to slosh the solvent the test tube must remain undisturbed

4. Observe the solvent movement and the separation band

5. When the pigments have separated into different bands lift the cork with paper attached from the test tube. Remove the cork from it

6. Allow the paper to dry completely

4.1.3 EXTRACTION OF PIGMENTS

1. Measure the distance from the pencil line to the solvent front and also the colour. Take a record of it

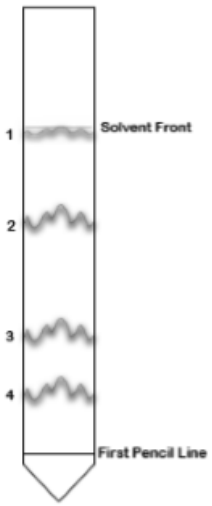
2. Cut each band of colour apart carefully and trim off the excess paper it should include all the pigment for each band

3. Label each test tube, one for each pigment

4. Cut each band of color into pieces small enough to fit into a 20-30 ml test tube. Insert the paper pieces into a 20-30 ml test tube. Insert the paper pieces in the appropriate test tubes

5. Add 5 ml of isopropyl alcohol to each test tube and seal with small piece of plastic wrap. Allow the samples to stand overnight

6. Calculate the R_f values by using the formula



4.2 DAY 2

4.2.1 PREPARATION OF SAMPLES FOR SPECTROPHOTOMETRIC ANALYSIS

1. Fill a cuvette half with isopropyl alcohol. Label it as Bl. This cuvette is used to standardize the spectrophotometer
2. In another cuvette transfer the solution from test tube containing pigment 1
3. More pigments require more cuvettes
4. Wipe the sides of cuvette with kimwipe and handel by the top edge to avoid fingerprints. The label should not interfere in the path of light beam

4.2.2 MEASURING ABSORBANCE OF PIGMENTS

1. Set the wavelength on the spectrophotometer to 360 nm and set mode to Absorbance by pressing the A/T/C button till an A is appeared on digital screen
2. Insert the blank into the cell holder and close the door.
3. Press the 0 ABS/100%T key to set the blank to 0 absorbance
4. Remove the blank
5. Insert the cuvette 1. And record the absorbance
6. Repeat the process with all pigments
7. Once all are finished do the same procedure with wavelength 380nm

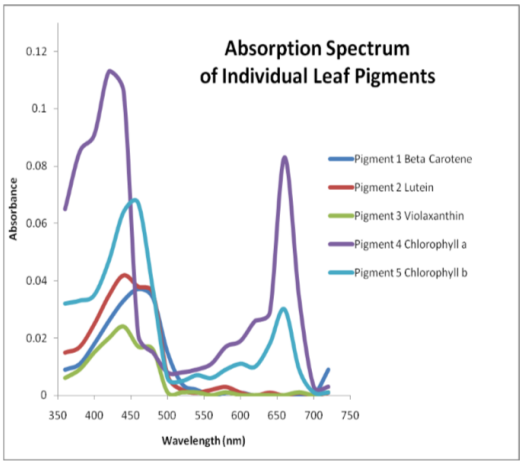
5. CONCLUSION

Rf Values:

- **β-carotene - 0.99**
- **chlorophyll a- 0.30**
- **chlorophyll b- 0.13**

- **violaxanthin - .40**
- **lutein- .68**

Wavelength	Pigment 1	Pigment 2	Pigment 3	Pigment 4	Pigment 5
360	0.009	0.015	0.006	0.065	0.032
380	0.011	0.017	0.009	0.085	0.033
400	0.018	0.025	0.015	0.091	0.035
420	0.026	0.035	0.020	0.113	0.047
440	0.033	0.042	0.024	0.106	0.064
460	0.037	0.038	0.017	0.021	0.067
480	0.034	0.036	0.016	0.015	0.037
500	0.015	0.007	0.001	0.008	0.006
520	0.004	0.002	0.001	0.008	0.005
540	0.002	0.001	0.001	0.009	0.007
560	0	0.002	0	0.011	0.006
580	0.001	0.003	0.001	0.017	0.009
600	0.001	0.001	0	0.019	0.011
620	0	0	0	0.026	0.010
640	0	0.001	0	0.029	0.018
660	0	0	0	0.083	0.030
680	0	0.001	0.001	0.034	0.009
700	0	0	0	0.003	0.001
720	0.009	0.001	0.001	0.003	0.001



References:

1) Diagrams and images – Google Images.
2) Most of the information by Google Scholarly Articles.