Chromatography Experiment

Title: separation of pigments of photosynthesis using paper chromatography.

Goal (main aim):

Calculating the R_f of every single pigment, in order to distinguish it and identify its solubility.

Hypothesis:

In photosynthesis; two types of pigments are involved; chlorophyll (A or B) and carotenoids, by using paper chromatography we can distinguish each pigment, by measuring the color's R_f; referring to the next formula:

$$R_f = \frac{\text{Distance between the starting point and pigment line}}{\text{Distance between the starting point and the solvent fonte}}$$

Variables:

Independent variable:

- Pigment solubility.

Dependent variable:

- The distance that the pigments move, on the chromatography paper.

Controlled variables:

VARIABLE	*WAY OF CONTROL*
Temperature	The experiment will be carried out in room temperature.
Solvent volume	In order to minimize uncertainty; the solvent will be added using a pipette.
Volume & concentration of	The extraction sample will be

extraction	taken using the same tweezers.

Materials and procedure:

Materials:

1- Chromatography Jar.
2- Plant.
3- Chromatography paper.
4- Solvent (organic).
5- Tweezers.
6- Pipette.
7- Mortar and Pestle.
8- Scissors.
9- Ruler.
10- Calculator.

Procedure:

- Preparation of the mixture:

1- Place a piece of the obtained plant leave into the pestle.
2- Add 5ml (approx.), of 90% isopropyl alcohol.

- Preparation of the chromatogram:

- Attain a chromatography paper.
- Cut the paper, in order to have a triangular end.
- Draw a line above the triangular end, with 1 cm, draw a point in the center of that line, the previous line is considered the start line.
- By tweezers, take an extraction sample, put in on the center of the start line.
- Wait until the pigments of the sample are absorbed.
- By a pipette, pour 1 mm (approx.) of the solvent, into the chromatography jar.
- Insert the chromatography paper (containing the pigments of the extraction), in the chromatography jar until the triangular

end is totally emerged by the solvent. (Keep in mind not to let the extraction pigments touch the solvent).

- Close the jar firmly, to prevent solvent evaporation.
- Wait, until the pigments start dissolving, as a result of the solvent elevating on the paper.
- When the solvent is 1 cm away from your paper's top, take the papers out and mark the farthest point of the solvent's elevation (solvent front). (Make sure you mark quickly before the line evaporates).
- Repeat the previous steps for more results.
- Calculate R_f using the following formula:

$$R_f = \frac{\text{Distance between the starting point and pigment line}}{\text{Distance between the starting point and the solvent fonte}}$$

- Put your results in the form of a table.
- Compare results with the standard R_f table.(This helps to determine the type of pigment obtained).

<u>Data collection and processing:</u>

Points of each color appearance	1st trial Solvent front=6 $\pm 0.05cm$		2nd trial Solvent front=6 $\pm 0.05cm$	
	d	R_f	D	R_f
X_1	2.5 $\pm 0.05cm$	0.42 ± 0.01	$3.5 \pm 0.05cm$	0.58 ± 0.01
X_2	4.8 $\pm 0.05cm$	0.80 ± 0.01	$4.0 \pm 0.05cm$	0.67 ± 0.01
X_3	4.9 $\pm 0.05cm$	0.82 ± 0.02	$5.8 \pm 0.05cm$	0.96 ± 0.02

<u>Table(1): experiment's raw data</u>

- (d): distance that the pigment moved.
- Uncertainty of solvent front:

$$\frac{minimum\,reading\,of\,the\,ruler}{2}$$

- The average of the R_f:

$$\frac{1st\,trial\,result + 2nd\,trial\,result}{2}$$

Points of each color appearance	1st trial Solvent front=6 $\pm 0.05cm$		2nd trial Solvent front=6 $\pm 0.05cm$		Average	The closest value in the standard R_f table
	D	R_f	D	R_f	R_f	
X_1	2.5 $\pm 0.05cm$	0.42$\pm$0.01	3.5$\pm 0.05cm$	0.58$\pm$0.01	0.50 $\pm$0.05	(0.45) Chlorophyll b
X_2	4.8 $\pm 0.05cm$	0.80$\pm$0.01	4.0$\pm 0.05cm$	0.67$\pm$0.01	0.70 $\pm$0.005	(0.65)Chlorophyll a
X_3	4.9 $\pm 0.05cm$	0.82$\pm$0.02	5.8$\pm 0.05cm$	0.96$\pm$0.02	0.89$\pm$0.02	(0.83)Phaetophytin

Table(2): experiment's quantitative results

Conclusion and evaluation:

Conclusion:

1- Photosynthetic pigments can be separated using paper chromatography, which is shown by results in table (1).
2- The average (R_f) of (X1) (0.50±0.05) and (X2)(0.70±0.05), can be considered close to the standard (R_f) (0.45) and (0.65) respectively, which shows that the method used to calculate (R_f) in the hypothesis is valid.

Evaluation:

- In table (1), a clear difference can be seen between the reults of the 1^{st} and 2^{nd} trials, and also between the average (R_f) of (X3) and the standard (R_f), which shows the occurrence of errors, those errors could be:
- To cut a triangular end, a pair of scissors was used; using a pair of scissors doesn't always guarantee the formation of an isosceles triangle, which affected the elevation (diffusion), of the solvent up the paper.
- Since tweezers were used to add the extraction samples, it had a huge possibility of uncertainty when it comes to the equality of the volume taken for the two trials.
- A ruler was used to measure the distance moved by each pigment, and this contains a pretty much big amount of uncertainty involved.