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An experimental study on plant pigments

Aim

The aim of this experiment is to divide and identify the different pigments found in a spinach plant by using paper chromatography.

Introduction

Paper chromatography is a practical and quick method to separate different coloured chemicals or substances. This technique has various uses, and in this case it can be used to separate and identify different plant pigments. Pigments are substances produced by living organisms that have a color that results from selective color absorption. Plants contain different types of pigments, and the most important are chlorophylls, carotenoids, anthocyanins and xanthophylls which absorb different varieties varieties of colours ranging from green to red to blue. With paper chromatography it will be possible to calculate the Retention Factor which is how soluble the particular pigment is, in comparison with the solvent.
A smaller Rf value usually indicates a larger and less soluble pigment, while a bigger Rf value tends to indicate a smaller and more soluble pigment. The formula for the Retention factor is: distance travelled by pigment / distance travelled by solvent.

Educated guess

With paper chromatography it is possible to calculate and identify most of the various types of pigments present in plant leafs.

Variables

Dependent variables	Independent variables	Controlled variables
Visibility of plant pigments	Quantity of spinach juice	Temperature of acetone
Distance moved by substances	Period of time immersed	Type of leaf
Distance moved by substances	Amount of acetone	Type of chromatography paper

Materials

- Mortar
- Pasteur pipette
- Ruler
- Acetone
- Spinach leaves
- Small backer
- Chromatography paper
- Pencil

Fig. 1 —> Smashing the spinach

Method

Using a mortar, grind the spinach leaves for some minutes, until the leaves will be completely shredded and at least a small amount of juice is created. Add some drops of acetone in the juice and then extract all the liquid by using a Pasteur pipette and deposit it inside a clean test tube. In the clean and dried becher pour approximately one centimetre from the bottom of acetone with a new pipette. Moving on, using the pipette previously used to pour the spinach and acetone juice in the test tube, pick up a discrete amount of juice. Gently pour the juice at around 2 centimetres from the bottom of the chromatography paper trying to create a proportionate line. Wait one minute to let the paper completely absorb the substance and then wave it for 10 second in the air to completely dry the chromatography paper. Place the paper into the backer and check that the substance is clearly on top of the solvent. Wait for ten minutes to let the chromatography run take place, and when the substances have clearly stopped moving, remove the paper from the solvent. With a ruler measure the distance travelled both by the solvent and by each of the different colours identifiable. Use the formula for the Rf value to calculate the retention factor and identify the different pigments. Repeat the experiment at least twice too be sure to be able to identify at least some pigments.

Fig. 2 —> Chromatography papers before immersions

Results

Color	Distance travelled by solvent	Distance travelled by pigment	Rf value calculation	Pigment detected
Light green 1	4.5 cm	1.5 cm	1.5 / 4.5 = 0.33	Xanthophylls
Light green 2	4.8 cm	1.4 cm	1.4 / 4.8 = 0.29	Xanthophylls
Dark green 1	4.5 cm	2.6 cm	2.6 / 4.5 = 0.57	Chlorophyll a
Dark green 2	4.8 cm	2.8 cm	2.8 / 4.8 = 0.58	Chlorophyll a
Orange 1	4.5 cm	2.1 cm	2.1 / 4.5 = 0.46	Chlorophyll b
Orange 2	4.8 cm	2.2 cm	2.2 / 4.8 = 0.46	Chlorophyll b

Discussion

Fig. 3 —> Chromatography paper inside solvent

The results showed that paper chromatography is a fast and reliable method to separate different substances to visualise and identify them. This is possible because chromatography paper is a type of paper that absorbs the solvent and makes it move up by capillary action. As the solvent crosses the area containing plant pigment extract, the pigments dissolve in and move upwards with the solvent. Chlorophyll is the strongest of the pigments, in fact especially in summer there is so much chlorophyll in the leaves the other pigment cannot be seen. For this reason the results would be more precise if the experiment is done using a leaf during winter, in this way chlorophyll would be present in much lower levels and the other pigments would stand out.

Conclusion

The educated guess which stated that with paper chromatography it is possible to calculate and identify most of the various types of pigments present in plant leafs was partially correct as generally the most important pigments present were identified, even though many of the others such as anthocyanins and xanthophylls were not clearly visible or visible on the chromatography paper.

Evaluation

The results of the experiment where coherent with what thought, so that not all pigments would have been identifiable. This issue could be solve in various different ways. Firstly by pouring the spinach and acetone juice on the chromatography paper in a more precise manner by using appropriate instruments and performing the process more slowly. Secondly the experiment could be repeated more times than twice, in this way, it will be more probable that all the pigments will be visible in at least one chromatography paper. Lastly, the experiment can be also improved by using a variety of different plants, as a pigment not clearly visible in the spinach may be more evident in a different type of plant.

References

- https://www.bbc.co.uk/bitesize/guides/zw8739q/revision/7
- https://www.scienceinschool.org/content/colour-chlorophyll-and-chromatography