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Biology; IB5

LAB REPORT #7
CHROMOTEMOGRAPHIC SEPERATION OF PLANT PIGMENTS

AIM:

My aim is to extract the various photosynthetic pigments from a grass tissue and later to separate them and determine which one is which by using thin layer chromatography.
To calculate Rf values for all of the pigments.

Hypothesis:
I expect to observe various pigments extracted. There will be a distinction between them and separation will occur due to the pigments' different solubility and attraction to the static phase.

Prediction:
I predict that separation of each of the pigments will occur and will be able to be observed. I also predict that the pigment that will travel the closest is chlorophyll b, and then chlorophyll a, followed by xantophyll and at the end there will be cartenoid pigments.

Variables:
There are no variables in this specific lab, because it is an observation lab, where we are meant to observe separation of pigments.

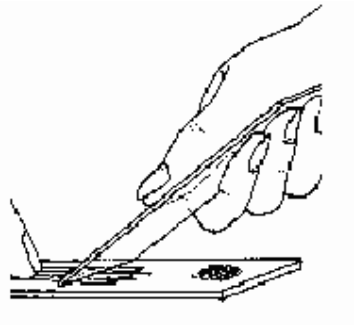
METHOD:

Equipment:

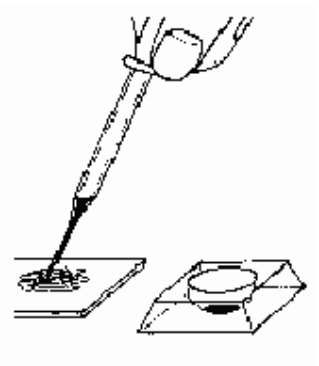
- A few grass leaves
- 2 glass microscope slides
- blu tac
- 1 glass micro pipette
- 10 cm^3 propanone (acetone)
- 1 small watch glass
- 1 electric hair drier
- 1 very fine paintbrush
- 2 – 3 TLC chromatography strips (1.25 cm x 6.7 cm)
- 1 glass specimen tube, (2 cm x 7.5 cm)
- 1 cork to fit the tube, with a horizontal V-slit
- 7 cm^3 chromatography running solvent per tube
- 1 pencil
- seeker (mounted needle)

Experimental procedure:
I would divide the whole experimental procedure into two parts: extracting the leaf pigment and separating them using chromatography. After all this process there must be observations and identification of the results made and the experiment is complete.

EXTRACATING THE LEAF PIGMENT:



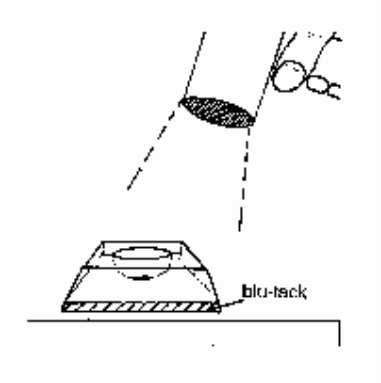
1) I placed 2-4 grass leaves on a slide and then used a second slide to scrape the juice out. I repeated the scarping a few times in order to collect as much of the juice and as concentrated as possible at the end of the slide. A dark "mush" collects at the end. I later removed any larger pieces of debris with a seeker (mounted needle).



2) I then tilted the slide above the watch glass and added around six drops of propane (acetone) to the green mush and mixed it well. After all that I trickled it all down into a watch glass.

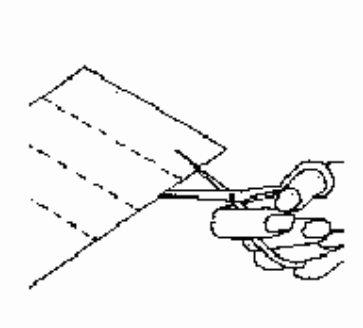
ATTENTION! ACETONE IS HIGHLY FLAMABLE!
KEEP AWAY FROM FIRE OF ANY KIND!!!

3) Repeat it all taking around 4 more grass leaves. Ensure to achieve having c. 20 drops of the extract in the watch glass.



4) Secure the watch glass to bench with Blu Tac and gently dry it with a hair-dryer (2-3min). It should remove all water from the extract.

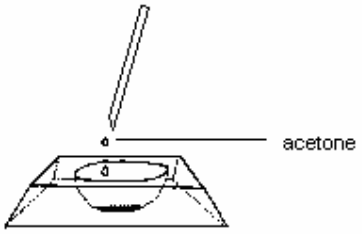
SEPARATING THE LEAF PIGMENT:



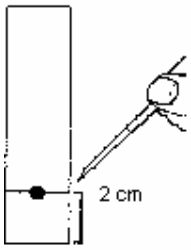
5) Prepare 2-3 strips of chromatography paper. Cut a TLC plate into small stripes e.g.1.25 x 6.7 cm, so that they fit your tubes. Try not to touch the surface of the plates.

6) Write your initials and source of sample at the top of the paper strip to ensure it doesn't get mixed up with some other results.

7) Draw a pencil line 2 cm from the bottom of the paper strip.



8) Add 2-3 drops (only) of acetone to the dried pigments in the watch glass and gently mix it with a wooden stick. We added this acetone in order to solve the pigments that were dried at the bottom of the bowl.

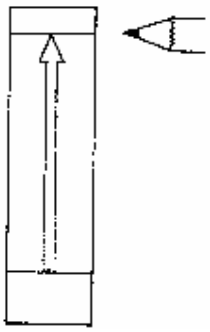


9) Transfer tiny amounts of this extract using a glass pipette to make a concentrated spot in the middle of the pencil line, which is 2 cm from the bottom. In order to make it as concentrated as possible, you must ensure yourself to put it in a very small point.

10) Dry it off with a few passes with a hair-dryer. Repeat around 10 times to get a dry concentrated spot.

11) Transfer your paper samples into a mobile phase and watch. At the bottom there is the solvent and just above it there is the spot done by the grass extract, which under no circumstances can touch the solvent. We are waiting now for the separation of the various pigments.

To prepare the active phase: we took a glass container and put in it a mixture of 10% acetone and 90% petroleum of 5mm height from the bottom. Then we placed the chromatographic paper in the glass container and made sure the pigments are above the solvent.



12) When the rising liquid (solvent front) is close to the top, remove the paper and mark any zones with color in a pencil.

13) Measure the distance run by the solvent front and by each of the pigments. All measurements should be made from the centre of the original spot to the front of each pigment spot.

DATA COLLECTION:

We haven't observed the pigments separation. They appeared to form a glut and concentrated only in one zone at the very top. They must have moved too fast and came to the very top too fast. Our results don't support the hypothesis.

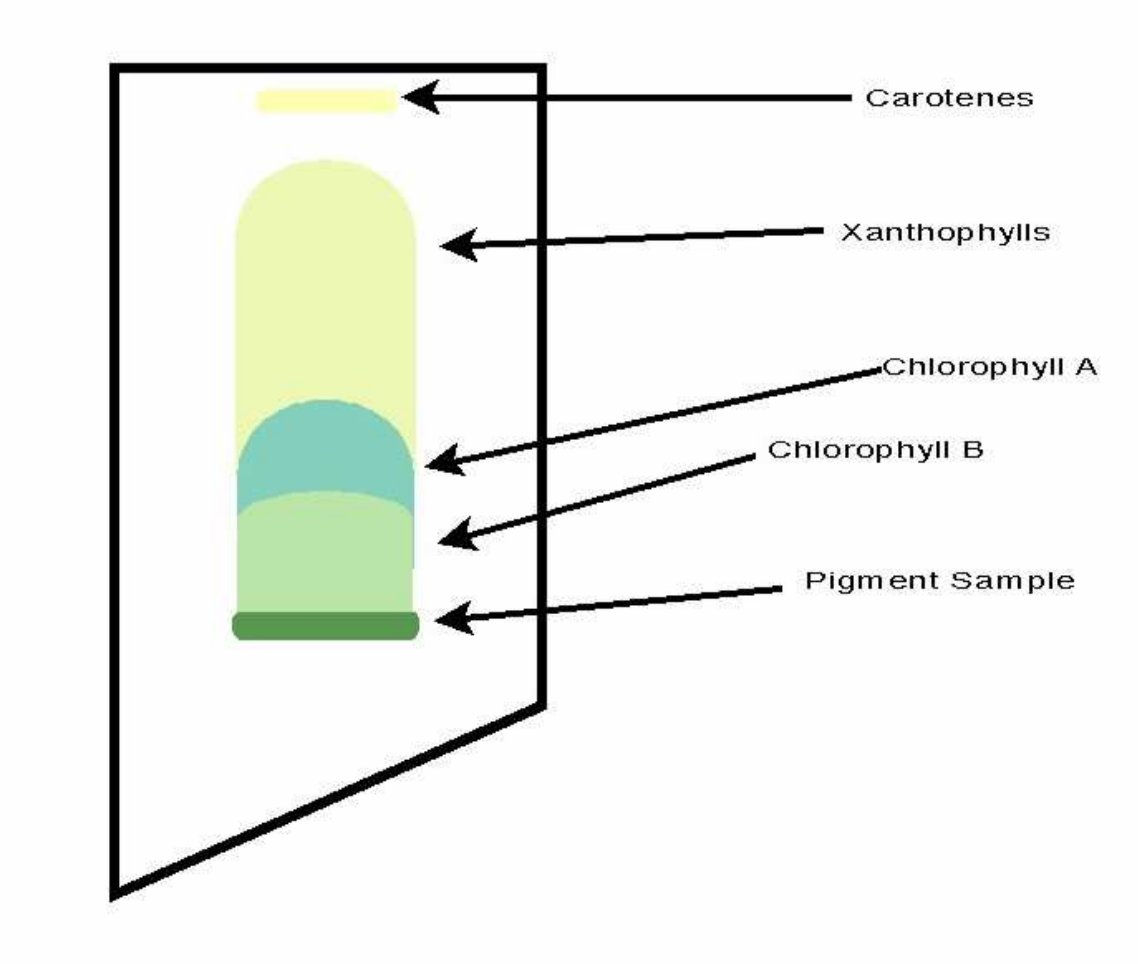
An Rf value is measured in order to represent the distance a pigment traveled in comparison to the solvent, which is also a representation of solubility of a specific pigment and their attraction to the static phase.

Rf = distance run by pigments / distance run by solvent

The Rf value from my experiment:

Pigment	Rf value	Color
Chlorophyll b	0.7	Dark green
Chlorophyll a	0.5	Pale green
Xantophyll	0.93	Yellow

Though, the other group got some very nice results, where a separation could be seen very easily. We achieved what we expected a lucid distinction between the pigments, where they moved different distances depending on which pigment we were looking.



The common, stated, official Rf values:

Pigment	Rf value	Color
Chlorophyll b	0.47	Dark blue-green
Chlorophyll a	0.65	Green
Xantophyll	0.71	Yellow-brown
Phaeophytin	0.86	Yellow-green
Carotene	0.96	Yellow

DATA ANALYSIS:

Chromatography has as its objective separation of molecules depending on their solubility (in this case it is the separation of various pigments).
In paper chromatography, when you place a colored chemical sample on a filter paper, you can get the colors to separate from the sample by placing one end of the paper in a solvent. As the solvent diffuses up the paper, it dissolves the various molecules in the sample varying on the polarities of the molecules and the solvent. If the sample contains more than one color - it has more than one kind of molecule. The differences in the chemical structures of each kind

of molecule, determine also their polarity, which will also alter while the structure alters. All that is going to lead to a different solubility in the solvent.

The unequal solubilities cause the various color molecules to leave solution at different places as the solvent continues to move up the paper. The more soluble a molecule is, the higher it will move up the paper.

Thanks to our experiment we can identify differences in polarity of pigment (color) molecules in grass.

Chroma means *color*; that's from wher *chromatography* got its name. However it is not the only chromatography, there various types, which not always involve color separation. In each, a mixture of molecules is drawn through a medium that separates the mixture by differences in polarity, solubility, molecular weight, or a combination of these factors.

What happened in our experiment is that the solvent migrated up the paper and eventually soaked all the paper, because its one end was put into a solvent. The extract from grass containing various pigments was also carried upwards with the solvent. The height a specific pigment migrates depends on how attracted the pigment is to the static phase and how soluble it is. If it is very soluble, the pigment will tend to move with the solvent front and the highest; if it is not too soluble it will lag behind the solvent and move little. In this way a complex chemical solution can be separated into its components according to their solubilities (polarities).
The various types of chlorophylls and carotinoids of plant photosystems are all membrane bound and therefore are only soluble in rather nonpolar solvents. These pigments have been removed from grass by an extraction into acetone and they were separated by us by chromatography by using a solvent of petroleum : acetone (9:1).

What I've observed is that carotene has moved the furthest, what means that it is the most soluble and the lightest and also has the smallest attraction to the static phase. In contrast chlorophyll b is the heaviest, the least soluble and most attracted to the static phase.
My Rf values show the same trend as the one's observed by scientists, what proves them right.

"Pigments are large and often complex molecules responsible for the different colors of plants and vegetables. The different variations of colors are due to combinations of pigments. The carotenoid pigments are responsible for orange, red, and brownish colors. Chlorophyll is important for plant photosynthesis. Carotene is important for proper vision."

As we see all the pigments are very important for biological life. Pigments have various peak of absorption in particular areas. Having a number of pigments id important for biological survival value, because light can be absorbed in all different wavelengths. It is central for photosynthesis, which is a biochemical process which converts light energy into chemical energy and facilitates life and growth. Photosynthesis has two stages – one light dependent and second chlorophyll dependent; during both of them water and carbon dioxide is converted into sugars, proteins and all life significant molecules.

Different wavelengths of light are captured by different photosynthetic pigments. Certain pigments absorb certain parts of the spectrum e.g. the chlorophylls – in the blue violet and the red parts. In order for light to be later used, it must be at first absorbed during the light -

dependent stage, when the pigments play a very important role – they absorb the light. The broader range of colors that absorb light is the higher the absorption will be.

EVALUATION:

Our group at the beginning got no results, meaning we got a concentration zone of the pigments at the very top. I suppose it might be due to the solvent's polarity.

Let's talk about the second results, though, where our hypothesis is supported, because we see the same pattern as expected and the separation of pigments occurred. The Rf values are alike the ones received by the specialists. The pigments move the further, the more soluble they are, the lighter they are and the less attracted to the paper they are. There of course were some weaknesses and a few modifications might have been done.

Weaknesses:

Our main weakness is that we didn't make any replicates, which would make our results more accurate and reliable. We should have done a few more paper tests using exactly the same method.
I feel as another weakness was that we didn't include any variables; we didn't even check how pigments of another plant would react.

Modifications:

The most important modification is to do replicates of the same experiment and at the end look on the average results.
We could have also experimented with different stationary and mobile phases e.g. aluminum foil and spray aluminum oxide.
We could have also looked on the experiment when using various solvents, which would highly affect the results.
We could have also left the paper for a longer/shorter time to observe the differences.
We should have ensured ourselves that the pigments were above the solvent, what is crucial, otherwise the pigments would be solved in the solvent. Maybe that is what happened in our first experiment.
We could have also tested different plants and check the differences – compare the results.

Sources:

Some internet sites:
http://www.grossmont.net/cmilgrim/Bio120/Lab/Paper_Chromotography_Results.htm
http://12.17.12.70/aai/committees/education/chromato.htm
http://www.mrs.umn.edu/~goochv/CellBio/labs/photosynthesislab/photo.html
http://www-saps.plantsci.cam.ac.uk/worksheets/project3.htm
http://www.nasaexplores.com/lessons/01-037/9-12_2.html
Biology book:
"Advanced Biology for You" by Gareth Williams
Class notes
Own knowledge