**Investigation using thin layer chromatography to separate photosynthetic pigments STUDENT**

**Introduction**

In this activity you will use thin layer chromatography to separate photosynthetic pigments extracted from plant tissues. Based on the distances moved by the pigments you will be able to identify the different pigments present.

**Aim**

To separate and identify photosynthetic pigments using thin layer chromatography.

**Intended class time**

* 1 hour

**Chemicals**

|  |  |
| --- | --- |
| Propanone (extraction solvent) | HSE warning symbol Highly flammable  Can cause serious eye damage |
| Chromatography solvent | HSE warning symbol Highly flammable  Can cause lung damage, drowsiness and dizziness  Harmful to the aquatic environment |

**Equipment**

* Thin layer silica gel chromatography plate
* Leaves
* Pestle and mortar
* Pencil
* Ruler
* Goggles
* Propanone (extraction solvent)
* Chromatography solvent
* Small filter paper
* Small filter funnel
* Test tube
* Micropipette and disposable tip
* Chromatography tank in fume unit, containing chromatography solvent to a depth of 0.5 cm
* Black polythene bag

**Health and Safety**

* Propanone (the extraction solvent) is highly flammable and can cause eye damage. Wear goggles and ensure there are no naked flames in the room throughout this activity.
* The chromatography solvents are harmful and highly flammable. Keep the lid on the chromatography tank as much as possible and keep the tank in the fume unit at all times. Avoid breathing the vapour. Wear goggles and ensure there are no naked flames **anywhere in the room** throughout this activity.

**Procedure**

*Note: for best results minimise the exposure of the pigments to bright light.*

1. Your teacher will tell you which plant species your leaf samples have come from. Make a note of this and put two or three leaves into a mortar. *Note: Larger leaves can be torn or cut into smaller pieces.*  
     
     
     
   **Plant species**………………………………………………………………………………………………
2. Just cover the leaves with propanone, taking care not to add too much.
3. Next, grind the leaves thoroughly with the pestle until a smooth pulp is obtained.
4. Examine the liquid in the mortar. If there is no free liquid, add a little more propanone. If the liquid is not dark green, add another leaf and grind it to a smooth paste.
5. Fold a filter paper, place it in a funnel and put the funnel in a test tube.
6. Pour the green pigment extract into the funnel, collecting the filtrate in the test tube. You only need a few drops.
7. Draw a line in pencil across a thin layer silica gel chromatography plate about 1 cm from the end.
8. Use a micropipette to transfer pigment extract to the TLC plate, creating a small spot in the centre of the pencil line.
9. Allow the spot to dry, then add another spot. Repeat until the spot is almost black, keeping it as small as possible (no larger than 2 mm diameter).
10. Put your TLC plate into the chromatography tank in the fume unit with the spot at the bottom. The solvent level should not reach as far up as the spot itself.
11. Cover the tank with a black polythene bag while the chromatogram runs. Lift the black bag every two minutes to check on progress of the solvent front.
12. When the solvent front is ̴2 mm from the top of the TLC plate remove the chromatogram, mark the position of the solvent front in pencil and leave in the fume unit for the solvent to evaporate. Be sure to replace the lid on the chromatography tank.
13. Mark the position of each coloured pigment in pencil. Draw a diagram, noting the colour of each pigment, or take a photograph of the chromatogram as some of the colours fade rapidly.
14. Measure the distance moved by the solvent front and the distance moved and colour of each pigment. Record this information in a suitable table.
15. Calculate the Rf value for each pigment and add these values to your table.

*Note: Rf = distance moved by pigment / distance moved by solvent front*

1. Using the information in the table below, try to identify the pigment spots you have observed.

|  |  |  |
| --- | --- | --- |
| **Colour** | **Rf value** | **Pigment** |
| Yellow | 0.95 | Carotene |
| Gray-brown | 0.83 | Phaeophytin |
| Yellow-brown | 0.71 | Xanthophyll |
| Blue-green | 0.65 | Chlorophyll a |
| Green | 0.45 | Chlorophyll b |

**Extension questions**

1. Why is a small spot of pigment needed on the TLC plate?
2. Why is propanone used as the extraction solvent?
3. Why is the chromatogram run in the dark?
4. How could you find out whether the separated pigment spots you have observed on your chromatogram are single, pure pigments or a mixture of two or more?

**To submit**

For this piece of work to count towards Practical Activity Group 6 of the Practical Endorsement, you should have recorded the measurements for the solvent front and the pigments, calculated the Rf values and then attempted to identify the pigments in the leaf extract. This should all be evidenced in a table. You should also include a diagram or photograph of your chromatogram. You also need to have considered the above questions as the answers to these will aid you in preparation for your written examinations.