Pn°2. Identification of photosynthetic pigments

1. Introduction

Plants are autotrophic living organisms, meaning that they can synthesise their own organic molecules using light as an important factor.

Light, which is a source of energy, is essential to the process of photosynthesis. This light energy is made possible by the presence of pigments. Pigments have the property of absorbing certain wavelengths, which gives them a specific colour.

Chlorophylls are the major pigments involved in photon capture. Chlorophyll is synthesised and degraded in the envelope of the chloroplast, but is only present and active as a pigment in the thylakoids.

Carotenoids always accompany chlorophylls in the thylakoid membranes, where they apparently play the dual role of accessory pigments for capturing light energy and photoprotectors against high light intensities. They are widespread in many plant tissues, including tomato parenchyma, carrot roots, petals and orange peel.)

2. Objective du TP

Extraction and separation of different photosynthetic pigments.

3. Materials and chemicals

Materials	**Chemicals**
- Mortar and pestle; - A test tube (25, 50, 100 mL); - Filter paper ; - Spinach leaves ; - A beaker (10 and 50 mL) ; - Erlenmeyer flask (10 and 50 mL); - Funnel ; - Pasteur pipette; - A magnetic stirrer ; - A rod and a bar; - A 2 cm wide strip of chromatography paper; - Aluminium foil ; - Parafilm.	- Sea sand ; - Petroleum Ether ; - Acetone (80%) ; - Dichloroethane ; - $CaCO_3$; - $CaCl_2$.

4. Methodology

a. Extraction of different photosynthetic pigments

1) Wash, dry and weigh 1.5 g of spinach leaves;
2) Cut the leaves into small fragments;
3) Add 4 ml of acetone and grind until a homogeneous mixture is obtained (in the presence of $CaCO_3$ and $CaCl_2$);

4) Add 6 ml of acetone and grind again;
5) Allow to settle for 10 min;
6) Collect the supernatant in an Erlenmeyer flask (filter on paper and collect the chlorophyll solution) and make up to 10 mL with acetone;
7) Close with parafilm and shake.

b. Separation of the different photosynthetic pigments by paper chromatography

The pigments are separated by paper chromatography using an apolar solvent : the pigments are separated according to their affinity for the solvent, i.e. the degree of apolarity of the pigments.

- Add 10 ml of migration solvent (petroleum ether/acetone/dichloroethane: 8.5/1/0.5 v/v/v) to the chromatography tube;
- Close the tube tightly;
- Using a pencil, draw a line 2 cm from the bottom edge of a strip of chromatography paper;
- Using a pasteur pipette, apply a thin strip of undiluted pigment extract to the line and dry (repeat the application and drying several times until a very dark stain is obtained);
- Place the chromatogram in the tube surrounded by black paper and close the tube;
- Stop the migration when the solvent front has reached 20 cm.
- Dry the chromatogram.

5. Requested work

- Draw the chromatography paper at the end of the experiment, indicating the distances travelled by the pigments and the solvent.
- Identify the pigments with the help of formulae, and justify their order of migration (See table below).

6. Results and interpretation

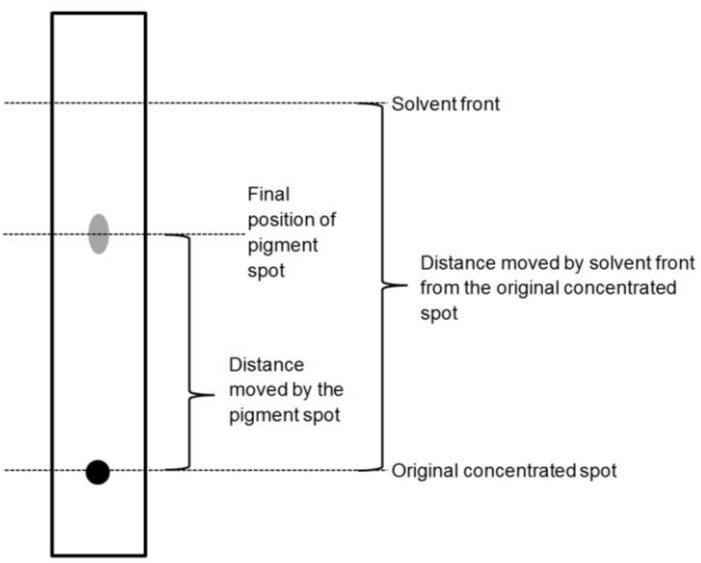

Figure 3: Measurements needed to calculate an Rf value

Formula for calculating the reference value

VR = distance crossed by pigment (mm) / distance crossed by solvent (mm)

VR : reference value (see table)

Table. Informations related to the identification of pigments

Pigment	Rf value range	Colour	Relative position
Carotene	0.89-0.98	Yellow-orange	Very close to the solvent front
Pheophytin a	0.42-0.49	Grey	Below the top yellow, above the greens
Pheophytin b	0.33-0.40	Brown	Below the top yellow, above the greens
Chlorophyll a	0.24-0.30	Blue green	Above the other green, below the grey
Chlorophyll b	0.20-0.26	Green-yellow	Below the other green
Xanthophylls	0.04-0.28	Yellow	Below, or almost at the same level of, the highest green

a) *β-Carotène*(C40H56) : Orange color;

b) *Chlorophyllea* (C55H72O5N4Mg): Green-blue color;

c) *Chlorophylleb* (C55H70O6N4Mg): Yellow-green color;

d) *Xanthophylle*(C40H56O2) : Yellow color.