EXTRACTION AND SEPARATION OF PHOTOSYNTHETIC PIGMENTS

- **OBJECTIVES**

- To extract photosynthetic pigments.
- To separate them by a simple paper chromatography technique.
- To recognize them by their properties: color and solubility.

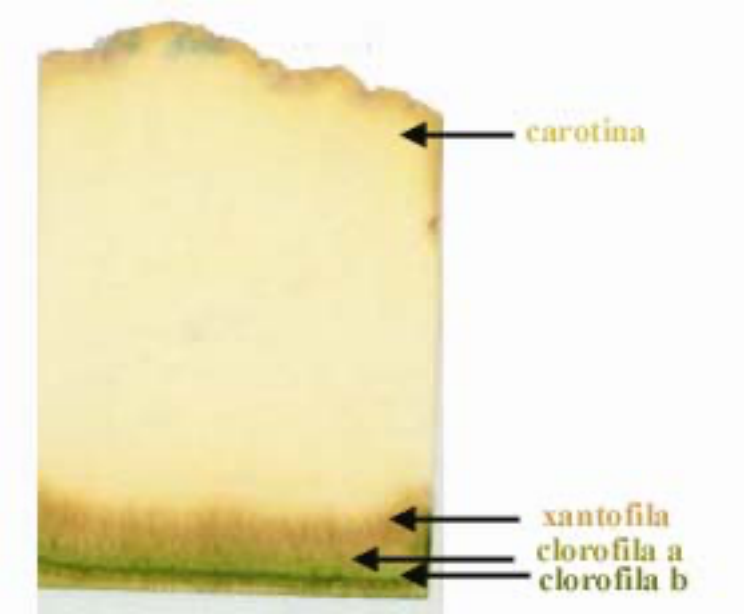
- **MATERIAL**
  - **Laboratory Material**
    - 2 beakers
    - Funnel
    - Mortar
    - Filter paper
    - Aluminum foil
    - Wooden clothespin (the one used for clothes) or a pencil
  - **Reagents**
    - 90° alcohol
    - Gasoline, benzene or acetone
    - $CaCO_3$.
  - **Biological material**
    - Spinach leaves

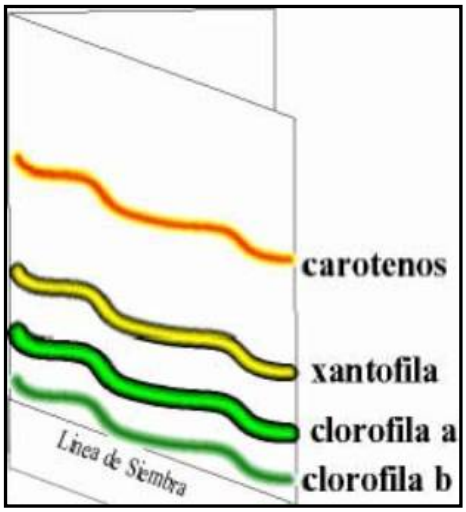
- **METHOD**

1. Wash the spinach leaves and remove the nerves.
2. Place the washed spinach leaves in a mortar and pestle and add 50 cc of 90° alcohol along with a small amount of CaCO3, which prevents the degradation of photosynthetic pigments.
3. Grind gently until the liquid has an intense green color.
4. Filter the liquid (with a funnel and filter paper) and collect the filtrate to obtain a crude solution of photosynthetic pigments.
5. Introduce the pigment mixture into a beaker and pour gasoline up to 1 cm in height.
6. Cut a strip of filter paper adapted to the size of the beaker and attach it to the beaker with a wooden clamp.
7. Insert the strip of filter paper in the beaker where you have placed the gasoline, in such a way that its end touches the bottom.
8. Cover the glass with aluminum foil. Leave the assembly like this for a few hours, the pigments will separate according to their absorption.

- **THEORETICAL BASIS**

When ascending the gasoline drags the pigments and, after a time (30-40 min.), four spots will have separated in the paper: a dark green one that corresponds to the **chlorophyll b**, above another green one that corresponds to the **chlorophyll a**, a yellow one that corresponds to the **xanthophylls** and a thin reddish stripe that corresponds to the **carotenes.**





- **ANALYSIS AND DISCUSSION OF THE RESULTS**

- Let the filter paper dry well and paste it in your laboratory notebook, point out and label each of the observed bands, indicating name and color of each pigment.
- Why is alcohol used to extract chlorophyll?
- How are the pigments separated, to which one of the color bands corresponds?
- Which are the most abundant pigments, which ones dissolve better in alcohol?