# Does "light" drive photosynthesis?

## Pigments in a spinach leave

#### Document: Separating pigments through chromatography

Chromatography, a physico-chemical analysis method, separates the constituents of a mixture by entrainment with a mobile phase along a stationary phase. The first chormatography was carried out in 1906 by the Russian botanist MikhailTswett (Михаил Семенович Цвет to his friends, 1872 - 1919), who separated the different pigments ("chromato" in Greek) in a spinach leaf: "Just like the light rays of a spectrum, the different components of a dye mixture unfold on the calcium carbonate column according to a law and can be analyzed qualitatively and quantitatively. "



#### Method:

- 1. Extract the pigments by crushing the leaves in a mortar, adding 10 mL ethanol and a pinch of sand. Filter and recover the extract.
- 2. Beforehand, pour the eluent into the tall beaker covered with a Petri dish lid, which serves as the chromatography tank. Wait 5 min before adding the silica plate once the deposition is complete, so that the eluent vapors have saturated the tank.
- 3. Draw the deposit line with a pencil about 1.5cm from the edge.
- 4. Use the plastic pipette to deposit the chlorophyll extract. Do not hesitate to place a drop, wait for it to be absorbed, then place a second drop. The spot should be fine, 5 mm in diameter. Caution: the extract deposit must not come into contact with the mobile phase.
- 5. Wait about 15 min when the migration front reaches 1.5 cm from the top edge of the plate.

#### Which different pigments can you identify?

Chlorophyl B (green/yellow)	Chlorophyl A (green/blue)	Xantophyl (yellow)	Carotenoïds (orange)
Weak migration			Strong migration

### What is the role of pigments in photosynthesis?

In 1882, German botanist Theodor Wilhelm Engelmann placed a filament of spirogyra, a freshwater green alga, in an environment rich in oxygen-hungry bacteria. He positioned the algal filament parallel to the width of the spectrum of decomposed white light illuminating it.



He made the following observation:



The absorption spectrum of a pigment corresponds to the quantity of light absorbed by the pigment vs. the wavelength of incident light. Wavelengths absorbed by the pigment are the ones used by the pigment.

The action spectrum of light corresponds to the efficiency of each wave length in a light-depending process.

Below are the absorption spectrum and the action spectrum of Chlorophyll A.



How do these spectra explain Engelmann's observations?