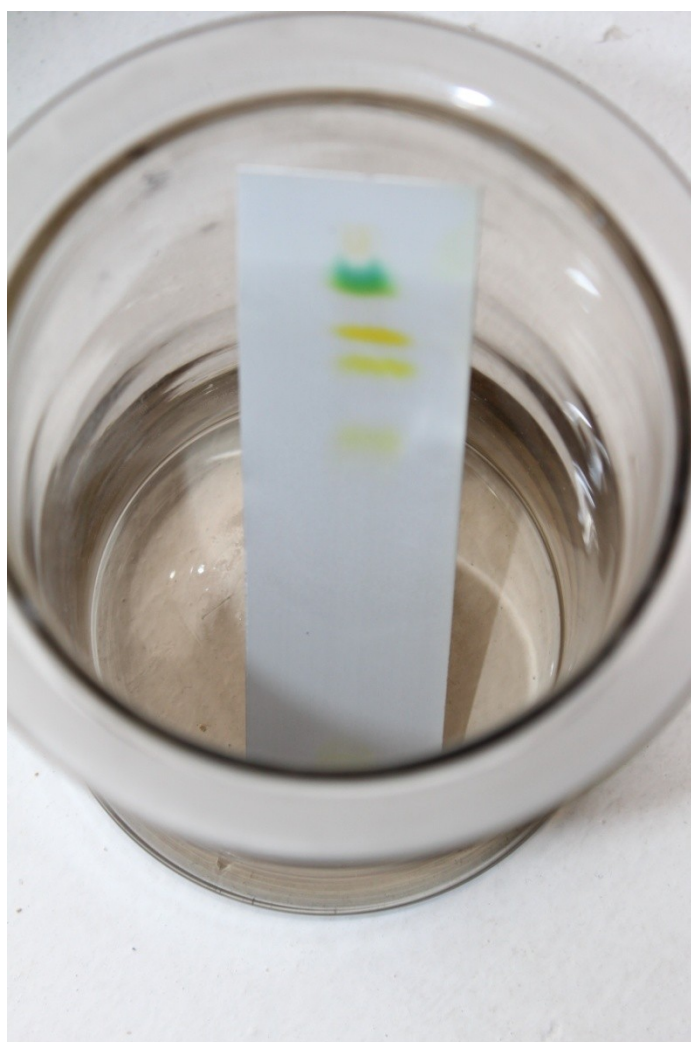


Separation of leaf pigments by thin-layer chromatography

Aim of the work

Students will separate the pigments in fresh spinach leaves. The four major plant pigments and their colors – chlorophyll a (medium olive green), chlorophyll b (blue-green), carotene (orange), and xanthophyll (yellow).



Materials needed

1. Eluent: diethyl ether-acetone in volume ratio 9:1.
2. Silica gel plates with dimensions 85 x 50 mm
3. Elution reservoir
4. Glass capillaries for spotting the samples.
5. Graduated test-tube.
6. Graphite pencil, ruler, scissors and rubber gloves.
7. Spinach leaf.

Preparing a spinach leaf pigment solution.

Place spinach leaves into the acetone, press by spoon the leaves down into the solution, cover the beaker tightly and allow to stay one day. Stir periodically and thoroughly macerate the spinach/acetone mixture to develop a thick liquid, after centrifugation upper layer store in freezer.

Procedure

1. Mark the starting line to the plate 1 cm from the edge of the plate - with graphite pencil.
2. The spot of spinach leaf extract are applied to the chromatographic plate.
Do not wet the silica beyond a diameter of 2-3 mm. After the liquid has evaporated (only a few seconds), add a second drop to the same spot. After application of samples let the spots dry. Pour 10 ml of eluent into the elution reservoir. Cover the chamber with lids and let the chamber atmosphere saturate with eluent vapours for 10 min. To start the analysis, insert the silica gel plate in reservoir and cover it with lid. Elution is stopped when the solvent front has traveled up the plate until 7-10 mm from the top of the plate.
3. Remove the plate from elution reservoir and quickly mark solvent front with a pencil.
6. Circle each colored spot with a pencil.
7. Measure the distance from the origin to the center of each colored spot and calculate the R_f values for all spots.

$$R_f = \text{distance traveled by amino acid} / \text{distance traveled by solvent}$$