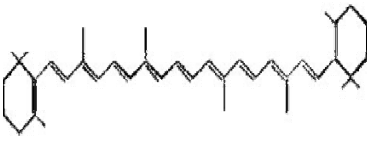
Identification of Plant Pigments (Carotenoids) by Thin Layer Chromatography

Introduction:

- Carotenoids are a class of natural fat-soluble pigments found principally in plants, algae, and photosynthetic bacteria, where they play a critical role in the photosynthetic process. They also occur in some non-photosynthetic bacteria, yeasts, and molds, where they may carry out a protective function against damage by light and oxygen. Although animals appear to be incapable of synthesizing carotenoids, many animals incorporate carotenoids from their diet. Within animals, carotenoids serve as antioxidants, and can be a source for vitamin A activity (Ong and Tee 1992; Britton *et al.* 1995).

- Carotenoids are responsible for many of the red, orange, and yellow colors of plant leaves, fruits, and flowers, as well as the colors of some birds, insects, fish, and crustaceans. Some familiar examples of carotenoid coloration are the oranges of carrots and citrus fruits, the reds of peppers and tomatoes, and the pinks of flamingoes and salmon (Pfander 1992). Some 600 different carotenoids are known to occur naturally (Ong and Tee 1992), and new carotenoids continue to be identified (Mercadante 1999).



Beta-carotene

Aim of the experiment:

-In this experiment you will extract plant pigments and then identify these pigments by chromatography .

Material:

- Petroleum ether
- Acetone
- NaCl (10%)
- $CaCO_3$
- Anhydrous Na_2SO_4
- Fresh leaves

- TLC chamber 22 × 22 × 10
- TLC silica gel plate
- Mortar & pestle
- Separating funnel 100 mL
- Measuring cylinder 100 mL
- 5 measuring cylinder 25 mL
- Erlenmeyer flask 100 mL
- Round bottom flask 100 mL

Procedure:

Developing solvent (mobile phase):

100 mL of petroleum ether, 11 mL of acetone and 5 drops of dist. water

Preparation of the TLC chamber:

The developing solvent is placed into a TLC chamber. The solvent should completely cover the bottom of the chamber to a depth of approximately 0.5 cm. The chamber is closed and shaken. It is kept covered so that evaporation doesn't change the composition of the developing solvent mixture. After 15 minutes the chamber will be saturated with the solvent vapor.

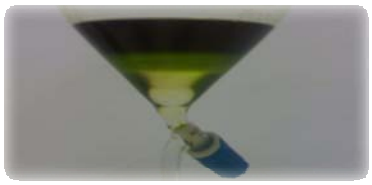
Extraction of the leaf pigments:

1- Using a pestle fresh leaves are grinded in a mortar containing 22 mL of acetone, 3 mL of petrol ether and a spatula tip-full of $CaCO_3$.

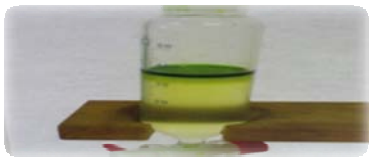
2- The pigment extract is filtered.

3- The filtrate is put into a separating funnel and is mixed with 20 mL of petrol ether and 20 mL of 10% aqueous NaCl solution.

The separating funnel is shaken carefully.



4- When the layers have separated the lower layer is allowed to drain into a beaker. This phase is thrown away.



5- The upper layer is washed 3-4 times with 5 mL of DW.

6- Afterwards the extract is placed in an Erlenmeyer flask and is dried with about 4 spatula tips of Na_2SO_4.

Filter and evaporate the solvent on water-bath until the final volume become of about 3 mL.

Application of the extract to the TLC plate:

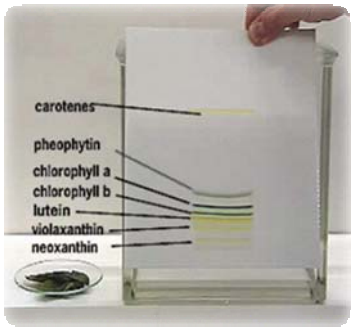
- With a pencil a line is drawn approximately 1,5 cm from the bottom of the plate. The procedure is repeated until the line is very dark green. The transferred extract is allowed to dry thoroughly after each addition. The line is kept as thin and straight as possible.

Experimental procedure:

- The loaded TLC plate is carefully placed in the TLC chamber with the sample line toward the bottom. The plate whose top is leaned against the jar wall should sit on the bottom of the chamber and be in contact with the developing solvent (solvent surface must be below the extract line). The TLC chamber is covered. The TLC plate is allowed to remain undisturbed. When the solvent front has reached three quarters of the length of the plate, the plate is removed from the developing chamber and the position of the solvent front is immediately marked.

Results and discussion:

- As the solvent rises by capillary action up through the TLC plate, the components of the pigment mixture are partitioned between the mobile phase (solvent) and the stationary phase (silica gel) due to their different adsorption and solubility strength. The more strongly a given component is adsorbed to the stationary phase, the less easily it is removed by mobile phase. The more weakly a component is adsorbed the faster it will migrate up the TLC plate. On the other hand, the running distance depends on the solubility of the pigment in the solvent. Since the experiment employs a high non-polar solvent (petroleum ether), the pigments that are least polar (carotenes) will be best solved in the non-polar solvent and will thus have the largest running distance.



R_f	leaf pigments	color
0.95	β-carotenes	golden
0.83	pheophytin	Olive-green
0.65	chlorophyll a	blue green
0.45	chlorophyll b	yellow green
	lutein	yellow
	violaxanthin	yellow
	neoxanthin	yellow
0.71	xanthophyll	Yellow-brown

References:

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Mercadante, A. (1999) New carotenoids: recent progress. Invited Lecture 2. Abstracts of the 12th International Carotenoid Symposium, Cairns, Australia, July 1999.

Ong, A.S.H., and E.S. Tee. (1992) Natural sources of carotenoids from plants and oils. *Meth. Enzymol.*, 213: 142-167.

Pfander, H. (1992) Carotenoids: an overview. *Meth. Enzymol.*, 213: 3-13.

Results Sheet