**Date ..... Name ..... Group .....**

## Instructions and lab report form for the practical lesson on biochemistry

### *Topic:* Separation techniques

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# Task 1: Separation of hemoglobin and potassium ferricyanide using gel filtration

**Objectives:**

- Measure absorption spectrum of ferricyanide to identify wavelength that can be used for its spectrophotometric determination.
- Construct calibration curve for ferricyanide.
- Explain why you cannot measure concentration of both analytes in unknown sample directly, using simple spectrophotometry? *(Check the absorption spectrum of both compounds.)*
- Separate both substances using gel filtration and determine their concentration.
- Determine elution volume of both compounds.

**Principle:**

**Observation/Results:**

Calibration:

Ferricyanide, λ=

$c_1$= A=
$c_2$= A=
$c_3$= A=
$c_4$= A=
$c_5$= A=
$c_6$= A=

**Conclusion:**

# Task 2: Thin-layer chromatography of plant pigments

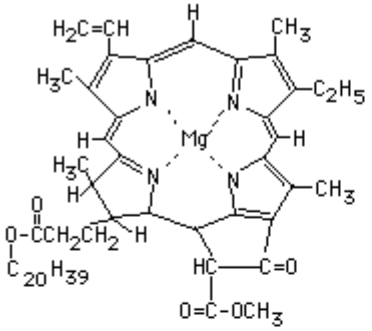
**Objectives:**

- Separate a mixture of plant pigments using thin-layer chromatography with two different mobile phases (highly non-polar hexane and a partially polar hexane: acetone mixture).
- Identify individual pigments on the chromatogram, developed in the partially polar mobile phase (your teacher will help you). Calculate Rf values for all pigments.
- Identify pigments on the chromatogram, developed in hexane (by yourself, without assistance from your teacher).
- Decide, which of the green spots belongs to chlorophyll a and which to chlorophyll b. Explain your conclusion. Refer to structural formulas of chlorophylls below.
- Highlight the difference(s) between lutein and β-carotene (by referring to the structural formulas below) and decide which one is more polar. Does it explain their different mobility in the partially polar mobile phase?
- Explain why most of the pigments do not separate in the non-polar mobile phase.

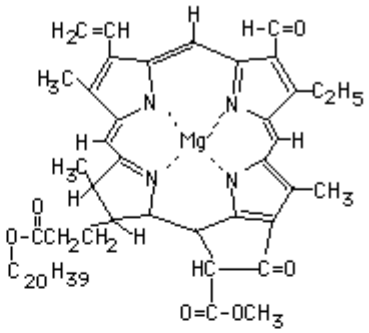
**Principle:**

**Observation:**

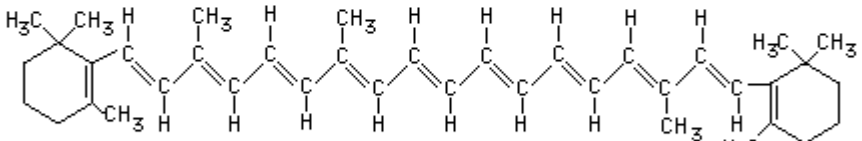
**Conclusion:**



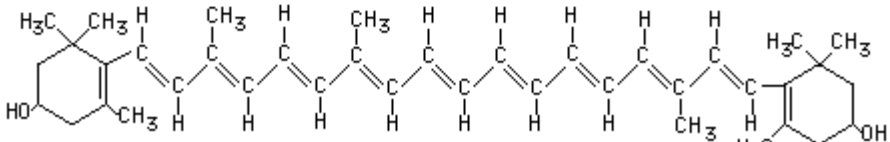
Chlorophyll a



Chlorophyll b



β-carotene



Lutein

## Task 3: Dialysis

**Objectives:** Demonstrate semi-permeability of dialysis membrane.

**Principle:**

**Observation:**

**Conclusion:**