

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:

- Separate both compounds using gel filtration and determine their concentration in unknown mixed sample.
- Construct elution curve and determine elution volume ( $V_e$ ) of both compounds. (Attach calibration graph and elution curve to the protocol.)

#### Principle:

#### Observation/Results:

Chosen wavelength:

Ferricyanide,  $\lambda =$

#### Calibration:

$c_1 =$              $A =$

$c_2 =$              $A =$

$c_3 =$              $A =$

$c_4 =$              $A =$

$c_5 =$              $A =$

$c_6 =$              $A =$

#### Calculations:

**Conclusion:** Why you could not measure concentration of both analytes in unknown sample directly, using simple spectrophotometry (and the gel filtration was a must)? (*Hint: Check the absorption spectra.*)

## **Task 2: Thin-layer chromatography of plant pigments**

### **Objectives:**

Separate a mixture of plant pigments using thin-layer chromatography with two different mobile phases (strongly non-polar hexane and partially polar hexane: acetone mixture). Describe and explain the differences in mobilities of individual pigments in both systems.

### **Principle:**

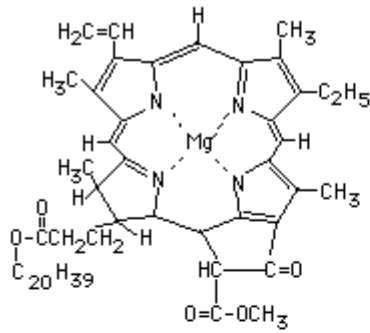
### **Observation+conclusion:**

Draw the position of individual pigments and identify them (your group teacher will help you with identification of pigments developed in hexane: acetone). Calculate R<sub>f</sub> for all pigments.

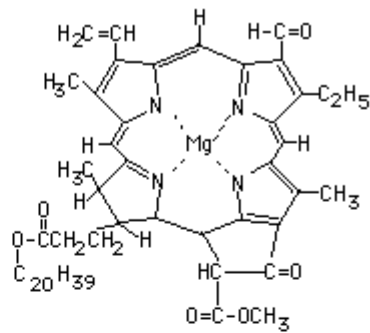
Decide, which of the green spots belongs to chlorophyll a and which to chlorophyll b. Explain your conclusion. (See structural formulas of chlorophylls below.)

Highlight the difference(s) between lutein and  $\beta$ -carotene (use structural formulas below) and decide which one is more polar. Does it explain their different mobility in the partially polar mobile phase?

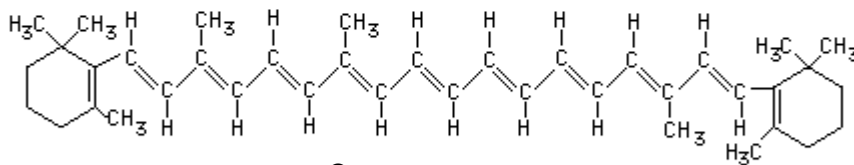
Explain why most of the pigments do not separate in the non-polar mobile phase.



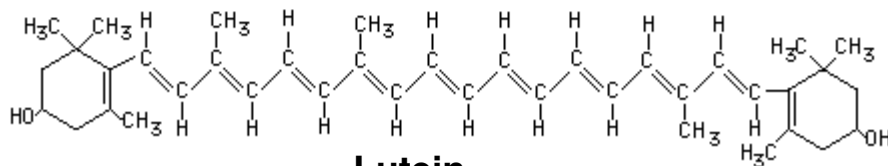
**Chlorophyll a**



**Chlorophyll b**



**β-carotene**



**Lutein**

### Task 3: Dialysis

**Objectives:** Demonstrate semipermeability of dialysis membrane.

**Principle:**

**Observation:**

**Conclusion:**