Surname and name ..... Group ..... Date .....

CHROMATOGRAPHIC TECHNIQUES FOR THE SEPARATION OF COMPOUNDS

1. Match the chromatographic term to its definiton:

● Adsorption ● Eluent ● Adsorbent ● Stationary phase ● Mobile phase ● Adsorbate ● Eluate

The carrier capable of interacting with the components of the mixture, does not move during the chromatographic process, it is usually solid or liquid	
The liquid or gas, which interacts with the components of the separated mixture, moves along the stationary bed in the chromatographic process	
Liquid used to elute components in the process of chromatography (especially in column chromatography)	
The liquid flowing from the column containing the separated, fractionated components of the mixture	
The process of binding substance molecules to the surface or interface of physical phases, causing local changes in the concentration of molecules.	
Stationary phase with adsorptive properties, on which the components of the mixture accumulate.	
Component of the mixture subjected to adsorption, accumulating on the surface of the stationary phase	

2. Choose a chromatographic technique that allows separation of mixtures…:

● Gel filtration ● Adsorption chromatography ● Ion exchange chromatography ● Affinity chromatography

substances with a different charge	
substances that differ in the polarity of functional groups	
substances that differ in their ligand binding ability	
macromolecules that differ in size	

3. Affinity chromatography. Haptoglobin is a glycoprotein which binds free hemoglobin in the blood. We want to isolate haptoglobin from biological material. Which of the following ligands should be immobilised on the agarose particles (stationary phase)? *(There is more than one option, select all of them)*

a) albumin
b) rabbit anti-haptoglobin antibodies
c) hemoglobin
d) lectin concanavalin A

5. The following protein mixture was separated by **gel filtration**: A - 10 kDa, B - 50 kDa i C - 160 kDa. In what order were these proteins eluted from the column?

4. Ion exchange chromatography. Plan how to separate the given protein mixture with an anion exchanger:
protein A: pI = 4.5; **protein B**: pI = 6.9; **protein C**: pI = 5.3

Give examples of anion exchanger functional groups ……..

What charge must proteins have to be bound by the anion exchanger?……...

Step 1. Column equilibration and protein binding:

Suggest the initial pH value: …….

What happens to the proteins on the column under these conditions? ……

Step 2. Increasing / lowering the pH of the eluent to …….. (*mark the correct one and suggest the pH value*)

Under these conditions, proteins are… (*underline*):

protein A bound / elute; **protein B** bound / elute; **protein C** bound / elute;

Similarly, plan Step 2 and possibly the next:

LABORATORY ACTIVITIES

Lab test 1. Thin layer chromatography of amino acids

Stationary phase (sorbent): ……..

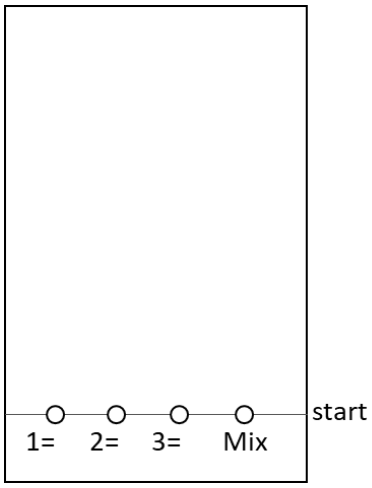
Composition of the eluting solution (eluent): ……..

Amino acid standards: ……..…….

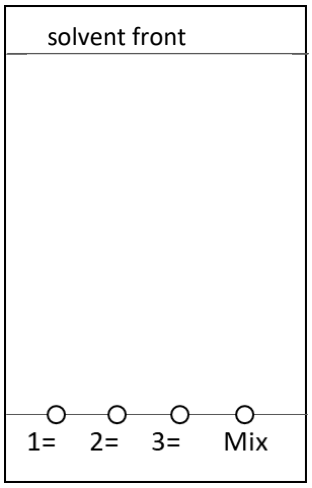
Procedure
Follow the instructions and/or the assistant's directions

1. Apply the samples to marked points on the starting line. Wait for the stains to dry.
2. Place the plate in the chromatographic chamber. DO NOT MOVE THE CHAMBER WHILE DEVELOPING THE CHROMATOGRAM!
3. After removing the plate from the chamber, mark the line of the solvent front with a pencil. Dry the plate well.
4. Spray the plate with ninhydrin reagent and heat it under an infrared lamp.
4. Measure distances: start-solvent front and start-center of the spot. Calculate the R_f values of the standard and sample amino acids (Mix). Identify the amino acids in the sample.

1. Initial TLC plate:
Scheme of sample application



2. TLC plate after separation:
Scheme of stains arrangement



3. Measure the distances traveled by:

Solvent front ……. mm;

Standard 1 ……. mm;

Standard 2 ……. mm;

Standard 3 ……. mm;

Mixture components (Mix):

Spot 1 ……. mm;

Spot 2 ……. mm;

Spot 3 ……. mm;

4. Calculate R_f values

Standard 1 ……. ; Standard 2 ……. ; Standard 3 ……. ;

Mixture components: Spot 1 ……. ; Spot 2 ……. ; Spot 3 ……. ;

How many components does the mixture consist of? ……..,

These are the following amino acids (*list*): ……..……

What feature of an amino acid determines the speed of its migration? ……..……

Lab test 2. Horse hemoglobin desalting by gel filtration

Stationary phase:……

Eluent: ……..……

The mixture to be separated:

……..…….

Procedure
Follow the instructions and/or the assistant's directions

1. Apply horse hemoglobin in 0.01M ammonium sulfate to the top of the column.
2. Elute it from the column with 0.9% NaCl solution.
3. Collect 15-20 fractions with a volume of about 2 ml: arrange in the order of flow, evaluate the intensity of the color.
4. Add 1-2 drops of Nessler's reagent to each fraction and re-evaluate the color intensity.
5. Draw the elution profile as a dependence of the color intensity on the elution volume/fraction number.

Were the components of the mixture completely separated from each other? Explain.

Compare the method of desalination by gel filtration with dialysis, indicate the advantages and disadvantages.

Lab test 3. Separation of organic dyes in adsorption chromatography on silica gel column

Adsorbent: ……

Eluent: ……..……

Adsorbate – the mixture of organic dyes:

……

Procedure
Follow the instructions and/or the assistant's directions

1. Apply organic dye mixture to the top of the column
2. Elute it from the column with methanol.
2. On the basis of the color of the successively collected fractions, observe the sequence of dyes elution from the column and evaluate the quality of the separation.
3. Draw the elution profile as a dependence of the color intensity on the elution volume/fraction number.

Were the components of the mixture completely separated from each other? Explain.

Which dye adsorbs the strongest and which the weakest on the column? Why? Try to explain the differences in the adsorption affinity of the separated compounds.