Student …….. Group …….. Data ……..…

I. SERUM PROTEIN AND LIPOPROTEIN ELECTROPHORESIS IN AGAROSE GEL

I. Serum protein electrophoresis is used to identify the presence of abnormal proteins, to identify the absence of normal proteins, and to determine when different groups of proteins are present in unusually high or low amounts in blood or other body fluids.

Proteins in gel are made visible by staining them with dyes
(Chapter 7, Lab test 1)

1. Electrophoresis conditions (fill in the gaps):

a. gel type and its concentration: ……..

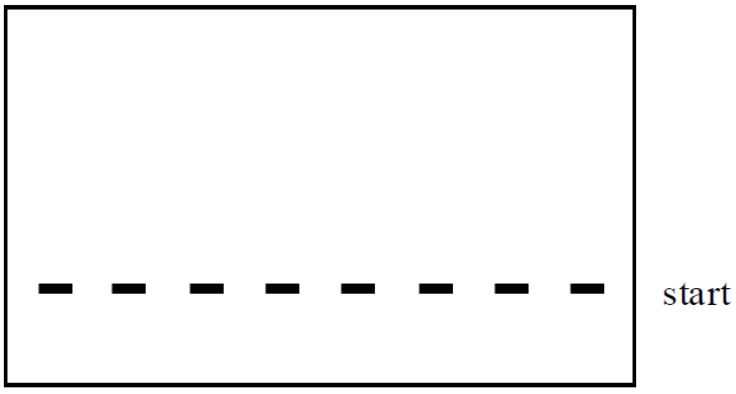
b. buffer pH = ……. overall charge of proteins: …….

c. voltage: ……..; separation time: ……..;

d. colour marker to monitor the process: …….

2. Scheme of applying samples (3 μl):

Mark the electrophoresis direction, name the electrodes and define their charges.



3. Post-electrophoresis treatment of plate

a. fixation; write down the composition of a fixative solution: …….

b. pressing, washing – write down the name and concentration of a washing solution: ……

c. pressing and drying

4. Gel staining – name a dye used for the staining:

a. specific – for all the serum proteins: _____

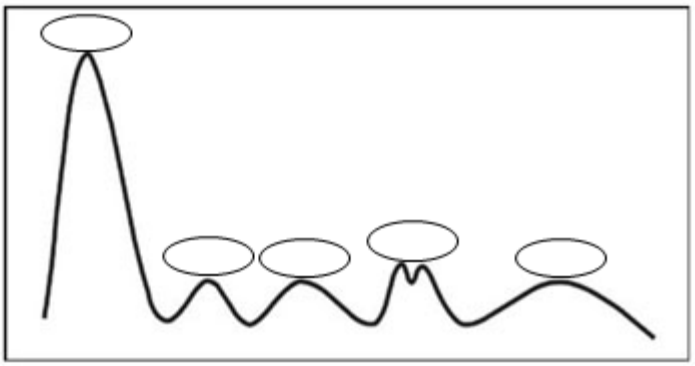
b. non-specific – for only lipoproteins: _____

5. Destaining of background - by washing with destaining solution (write down its composition):

…….

6. Evaluation of a protein fraction

Electropherogram – a visualisation of human serum proteins electrophoresis;
Densitogram – a diagram obtained as a result of protein fractions' optical density evaluation using a densitometer.



Mark protein fractions on both, electropherogram and densitogram.
Mark the electrophoresis direction, name the electrodes and define their charges.

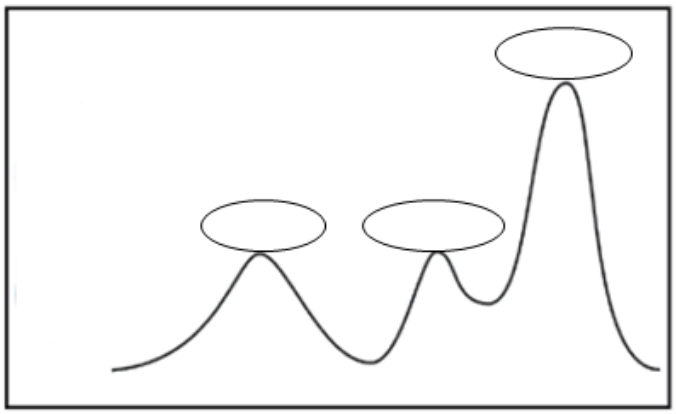
Write down the normal percentage ranges for each protein fraction:

The normal range of total protein in human serum is _____ g/dL or _____ g/L

Because disease states affect the relative amounts of albumin and globulin, the A/G ratio may provide a clue as to the cause of the change in protein levels

The normal albumin/globulin ratio is _____

II. Below you can see the result of normal **serum lipoproteins electrophoresis**: electropherogram on the left and densitogram on the right.



Mark lipoprotein fractions on both, electropherogram and densitogram.
Mark the electrophoresis direction, name the electrodes and define their charges.

Write down the normal percentage ranges for each lipoprotein fraction:

Total cholesterol in your blood includes both low-density lipoprotein (LDL) cholesterol and high-density lipoprotein (HDL) cholesterol.
The acceptable total cholesterol concentration in your blood should be less than _____ mg/dL

The borderline of total cholesterol concentration for adults is: _____ mg/dL

Name and surname …….. Group …….. Data ……..…

II. SEPARATION OF BIOMOLECULES. CHROMATOGRAPHY

1. Desalting a hemoglobin preparation. Gel filtration

(*Chapter 9, Lab test 2*)

Column matrix: ……

Eluent: ……

Analyte mixture: ……

Aim of the experiment: ……

Procedure.

a. Layer a mixture of horse hemoglobin contaminated with ammonium sulfate onto the top of the column.
b. Elute from the column with 0.9% NaCl solution.
c. Collect 15-20 fractions with a volume of approx. 1 ml. Arrange the collected fractions in the order in which they flow out. Assess the color intensity of the fractions.
d. Add 2 drops of Nessler's reagent to each tube and re-evaluate the color intensity of the fractions.
e. Plot the elution profile as the dependence of the color intensity (or absorbance, A) vs. the eluent volume or fraction number.

Observations and conclusions:

a. evaluate the efficiency of the protein desalination proces:

b. Compare the desalination method with dialysis:

c. Complete the following sentences:

Gel filtration is classified as: adsorption / partition chromatography (*choose the correct statement*)

Stationary phase is formed by …….

Mobile phase is ..…….

Separation is based on different …… of mixture components

…… is moving slowly because ……..

……..

2. Separation of a dye mixture. Adsorption chromatography

(*Chapter 9, Lab test 1*)

Column matrix: ……

Eluent: ……

Analyte mixture: ……

Aim of the experiment: ……

Procedure.

a. Follow the instructions in the manual and / or the teacher's instructions.
b. Collect fractions: taking into account the color intensity distribution, evaluate the quality of the separation and the order of the dyes eluted from the column.
c. Explain differences in adsorption affinity of separated compounds
d. Draw the elution profile of the separation as the relationship of color intensity (or absorbance, A) vs. eluent volume / fraction number)

Are the components of the mixture completely separated from each other?

How can the result be improved?

Which dye absorbs the strongest on the column and which the least? Why?

Adsorption chromatography is a separation method based on differences in: (*mark true statements*)

A. size of mixture molecules;
B. charge of mixture particles;
C. biological affinity of the mixture molecules to the ligand bound to the stationary phase;
D. the strength of physical interaction of molecules with a solid phase inversely proportional to the ambient temperature
E. the strength of physical interaction of molecules with the solid phase inversely proportional to the flow rate
F. polarity of the mixture molecules
G. solubility of molecules in a system of two immiscible liquids, e.g. water-ether