

Methods for protein fractionation

Task 1. Protein separation by use gel filtration method (gel chromatography) and column filled with Sephadex G-50

The aim of the task is to separate the blood plasma globulins from the salt $(\text{NH}_4)_2 \text{SO}_4$, which has been previously used for desalination of these globulins.

Protocol. Add 1 cm³ of blood serum and 1 cm³ of saturated solution of ammonium sulphate to a plastic tube. Mix and wait 5 min until a precipitate is formed. Next, centrifuge the tube using the force 2000 x g during 10 min. Remove gently the supernatant, add 1 cm³ of 0.9% NaCl solution (physiologic solution) to obtained precipitate and mix.

In the meantime, prepare three double-row tracks of glass tubes and mark row A and row B. Add 0.5 cm³ of copper reagent to row of tubes A, and 0.5 cm³ of BaCl₂ solution to the row B.

Apply the obtained solution of globulins into chromatographic column.

Chromatographic column handling

1. Put the bottle with physiologic solution (the mobile phase) on the shelf above the column on such height, that the eluent flow with a speed of 1 drop per second.
2. Turn off the "exit" tap of the column.
3. Disconnect carefully silicone tubing and needle.
4. Load the solution of protein onto the column by connecting the syringe with the needle
5. Turn on the "exit" tap of the column, and allow to sink the solution of globulins into the gel. Do not allow air to enter the gel!
6. If the globulin solution will be aspired to the gel, turn off the tap.
7. Replace the syringe by silicone tubing
8. Turn on the "exit" tap and start to sample the eluent to previously prepared glass tubes

Collect the eluent by sampling each 10 drops to glass tubes prepared previously in such a manner that the first fraction of eluent (first 10 drops) will be collected to the tube 1A containing



copper reagent, next fraction to tube 1B containing BaCl_2 , and continue collecting of eluent fractions respectively - 2A, 2B, 3A, 3B. Observe, in which tube with copper reagent the colour will change to violet (positive effect of biuret test), and in which tubs containing BaCl_2 a white precipitate will be formed (positive result of sulphur detection). Continue the chromatographic process up to the time when the eluent collected to tubs with BaCl_2 will not give any precipitate.

Preparation of the results

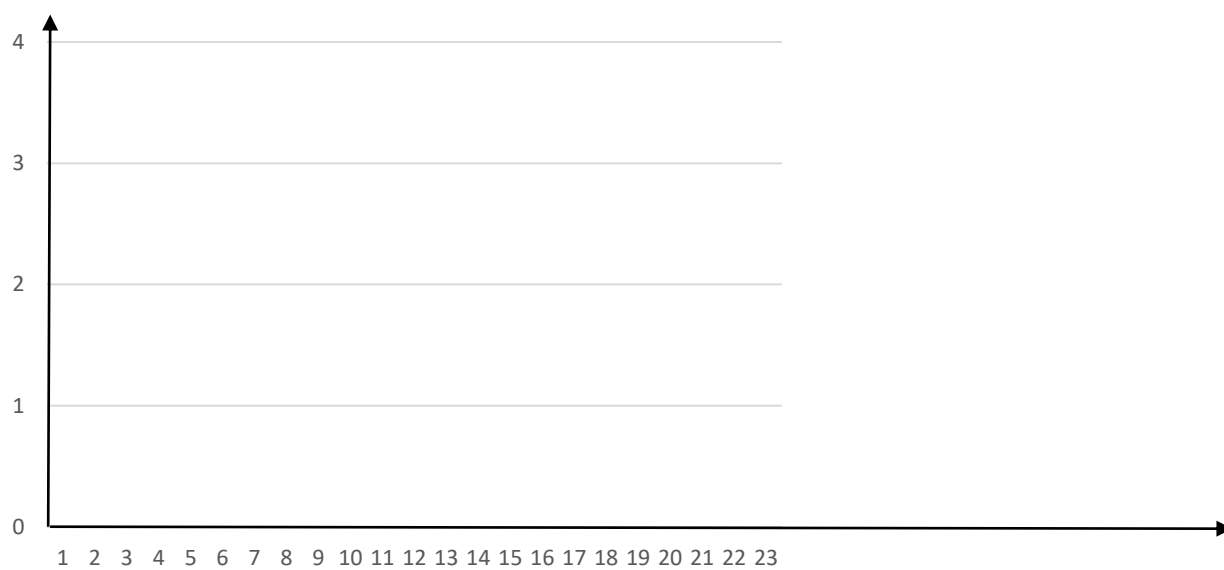
Fill in the table, using the scale:

- 0 - lack of colour or precipitate
- 1 - weak colour or precipitate
- 2 - visible colour or precipitate
- 3 - strong colour or precipitate

Fraction No	Biuret test	Precipitation test	Fraction No	Biuret test	Precipitation test
1, 2			41, 42		
3, 4			43, 44		
5, 6			45, 46		
7, 8			47, 48		
9, 10			49, 50		
11, 12			51, 52		
13, 14			53, 54		
15, 16			55, 56		
17, 18			57, 58		
19, 20			59, 60		
21, 22			...		
23, 24					
25, 26					
27, 28					
29, 30					
31, 32					
33, 34					
35, 36					
37, 38					
39, 40					

Plot the graph of the results of biuret and precipitation tests in following fractions.





Lublin, 10.03.2018

