INTERNATIONAL BIOLOGY OLYMPIAD e. V. IBO

IBO 2024 Kazakhstan

Q1-1

English (Official)

35th International Biology Olympiad

General Instructions:

The biochemistry practical exam consists of parts:

- Part I: Quantifying protein purification efficiency **(50 points).**
- Part II: Identifying compounds based on their titration curves **(50 points).**

Keep in mind:

- An answer sheet will be provided. **Only the answers provided in the answer sheet will be scored.**
- Check the equipment and materials provided according to the table below. If anything from the table is absent, please raise your “Other Queries **(Red)**” card.
- If you need water, please raise the “Drinking Water **(Blue)**” card.
- If you need to use the washroom, please raise the “Washroom **(Yellow)**” card.
- If you have any other questions, please raise the “Other Queries **(Red)**” card.
- The **"Spectrophotometer"** card will be used in the Part I.
- At the end of the exam, you will hear a signal. Stop answering and put down the pen immediately.
- Materials, papers, or equipment should not be taken out of the examination room.
- If you spill any liquid on your skin, please immediately notify the lab assistant by raising the "Other queries **(Red)**" card.
- Keep in mind that all consumables (tubes, reagents, 96-well plates, and tips) will not be provided or replaced additionally.

Drinking water card	Washroom card	Spectrophotometer card	Other queries card
			

Equipment and Materials

General:

Name	Quantity
Drinking water (Blue) card	1 piece
Washroom (Yellow) card	1 piece
Other queries (Red) card	1 piece
Safety goggles	1 pair
Pencil	1 piece
Ruler	1 piece
Yellow container for solid waste (labeled as "SOLID WASTE")	1 piece

Part I:

Name	Quantity
Spectrophotometer card	1 piece
Micropipette (100-1000 µL)	1 piece
Micropipette (20-200 µL)	1 piece
Micropipette (2 -20 µL)	1 piece
Tips (100-1000 µL)	40 pieces
Tips (2-200 µL)	40 pieces
1.5 mL tubes	20 pieces
$dH2O$ (Distilled water)	1 tube (1.3 mL)
96-well plate	1 piece
1xDye Reagent (labeled as "$1xDye\ Reagent$")	1 tube (12 mL)
Sample 1 (labeled as "$Sample\ 1$")	1 tube (1.5 mL)
Sample 2 (labeled as "$Sample\ 2$")	1 tube (1.5 mL)
2mg/mL BSA (Bovine serum albumin) stock (labeled as "$2mg/mL\ BSA$")	120 µL
Aluminum foil	1 piece

Part II:

Name	Quantity
50 mL burette filled with standardized NaOH solution	1 piece
25 mL serological pipette	3 pieces
Beaker for Compound 1 (labeled as "COMPOUND 1")	1 piece
Beaker for Compound 2 (labeled as "COMPOUND 2")	1 piece
Magnetic stirring bar	2 pieces
Magnetic stirrer	1 set
pH meter with an electrode probe	1 set
Electrode storage solution (labeled as "ELECTRODE STORAGE")	1 beaker
Squeeze bottle (labeled as "WATER") with water to rinse the pH meter probe	1 bottle
Beaker for liquid waste (labeled as" LIQUID WASTE")	1 piece
Pipette aid (Stripettor Ultra)	1 piece
Tissue papers (Kim wipes)	1 box
Retort stand with clamps	1 set
Compound 1 solution (labeled as "Compound 1")	1 tube (25 mL)
Compound 2 solution (labeled as "Compound 2")	1 tube (25 mL)

Introduction

Stomach cancer is one of the most prevalent cancer types in Kazakhstan, accounting for the death of approximately 10.1% of all cancer patients (World Health Organization, Global Cancer Observatory data, 2022). Most stomach cancer patients have an overexpressed oncogene A. It is known that the protein A (the protein product of oncogene A) has an enzymatic activity, which is critical for cancer growth. Researchers at Dulaty Taraz Regional University designed four candidate small-molecule compounds, Compound 1, Compound 2, Compound 3, and Compound 4 to inhibit the enzymatic activity of protein A and therefore inhibit cancer growth. The exact mechanism of action of these candidates is currently unknown. Still, it is believed to bind the critical allosteric surface of protein A and interrupt the catalytic cycle of protein A.

To test the efficacy of these compounds in targeting stomach cancer, researchers decided to use caged protein nanoparticles for drug delivery (Figure 1.1). Caged protein nanoparticles are hollow nanometer structures with well-defined geometries, consisting of self–assembled protein subunits. The space inside these nanostructures can be used as a reservoir to carry pharmaceuticals (Hong et al. Pharmaceutics 12.7 (2020): 604.)

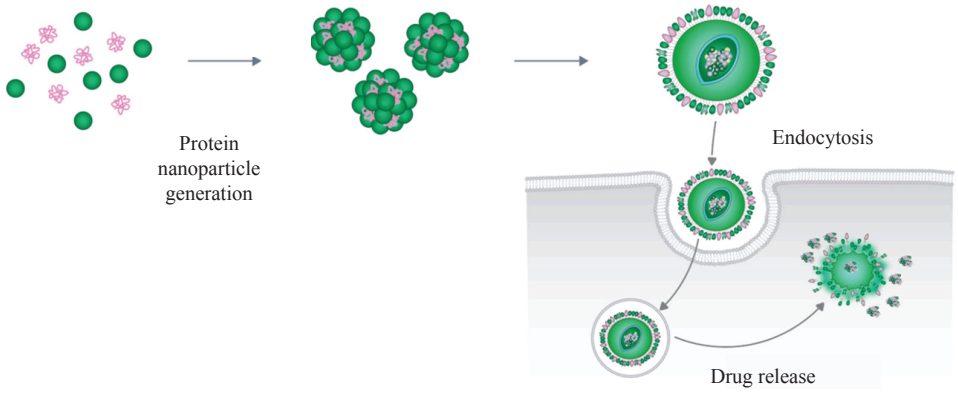


Figure 1.1. Scheme of drug delivery using protein nanoparticles.

Biochemistry

IBO 2024 Kazakhstan

Q1-5

English (Official)

Part I: Quantifying protein purification efficiency

Researchers at Dulaty Taraz Regional University used SUB1 as the subunit for their caged nanoparticle. To assemble caged nanoparticles, it is necessary to purify SUB1. To find the best purification method, researchers purified SUB1 with different strategies. Your task is now to quantify the concentration of SUB1 in these purified samples and determine the method that produces the greatest yield of SUB1.

To determine the concentrations of purified samples, you will use a Bradford assay, a colorimetric assay based on an absorbance shift of the protein-binding dye "Coomassie brilliant blue G-250".

Protocol:

Very important: Wear a lab coat, gloves, and safety goggles throughout the **ENTIRE** exam. **Note**: wearing safety goggles is optional if you already wear glasses for vision.

A. Label 8 tubes according to the first column of **Table 1.1** (1-8).

B. Prepare dilutions of BSA as shown in **Table 1.1.** Keep in mind that the Source of BSA for tubes 1, 2, 3 is the given stock solution, while the source of BSA for tubes 4, 5, 6, 7 are tubes 2, 3, 5, 6, respectively. Mix well by pipetting.

Tube #	**The volume of $\mathrm{dH2O}$ in µL**	**Volume of BSA in µL and (Source of BSA)**
1	-	25 (stock)
2	13	39 (stock)
3	25	25 (stock)
4	25	25 (Tube 2)
5	25	25 (Tube 3)
6	25	25 (Tube 5)
7	25	25 (Tube 6)
8	25	-

Table 1.1: Dilution scheme to prepare the standard curve.

C. Mix $\mathrm{1xDye\ Reagent}$ by inverting the tube a few times before use. Note: "1x" means you use it directly for mixing without diluting it.

D. Label 10 additional tubes according to the first column of **Table 1.2** (A-J).

E. Make solutions from Source (tubes 1-8, $\mathrm{Sample\ 1}$, $\mathrm{Sample\ 2}$) and $\mathrm{1xDye\ Reagent}$ as shown in **Table 1.2** in tubes A-J, respectively. Then gently mix the solution using a pipette.

	Source	Volume of source (μL)	1xDye Reagent (μL)	Total volume (μL)
Tube A	Tube 1	18	900	918
Tube B	Tube 2	18	900	918
Tube C	Tube 3	18	900	918
Tube D	Tube 4	18	900	918
Tube E	Tube 5	18	900	918
Tube F	Tube 6	18	900	918
Tube G	Tube 7	18	900	918
Tube H	Tube 8	18	900	918
Tube I	Sample 1	18	900	918
Tube J	Sample 2	18	900	918

Table 1.2: Preparation of samples for the Bradford assay.

F. Transfer the solution from each tube to the designated location on a 96-well plate as shown in **Figure 1.2**. Each well should contain 255 μL of solution from the corresponding tubes A-J (**Hint**: you can use 1 tip for triplicate wells). For example, wells B2, C2, and D2 should each contain 255 μL from tube A. Avoid making bubbles in the solution in the well.

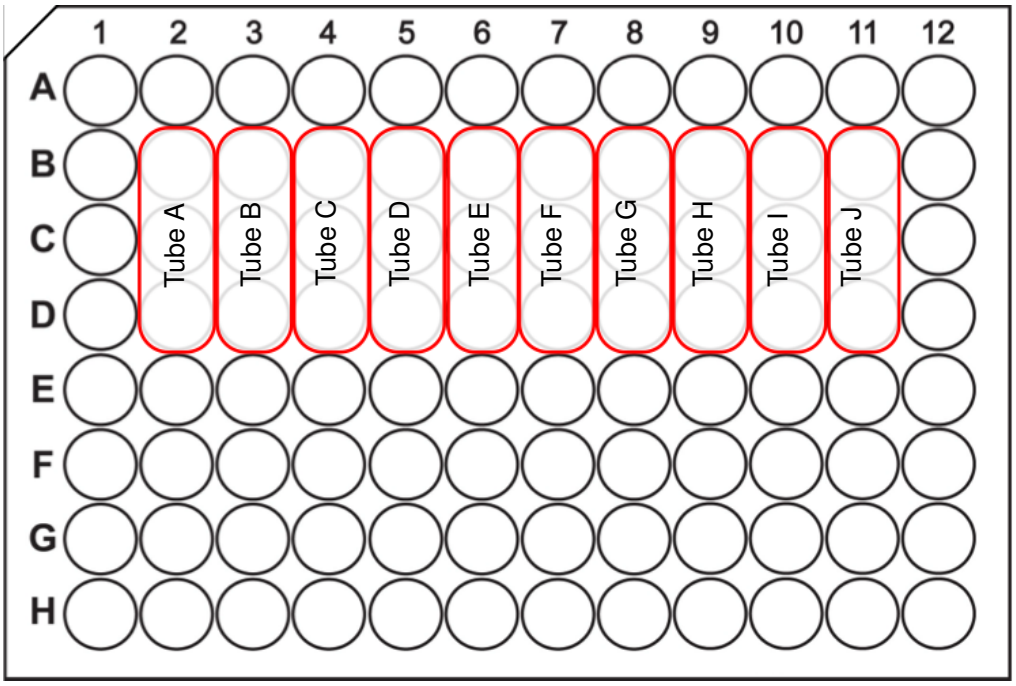


Figure 1.2. Designated location on a 96-well plate.

Question 1.1. After finishing step F, cover the plate with aluminum foil. Raise your Spectrophotometer measurement card (Spectrophotometer image) and pass the prepared 96-well plate to the assistant, who will measure the absorbance. 35.0pt

Note: you will not receive the measurements results back and they will be assessed separately. From now on, you will not perform any experiments for the Part I. You only need to answer the following theoretical Question 1.2, Question 1.3, Question, 1.4.

Question 1.2. In the Answer Sheet calculate and write down the final protein concentrations (rounded to the nearest integer in µg/mL) in tubes prepared for the standard curve. 6.0pt

Question 1.3. Using the equation in the standard curve prepared by researchers **before the examination** (**Figure 1.3**), calculate the average absorbances (rounded to three decimal places) of Sample 1 and Sample 2 and the protein concentrations (rounded to two decimal places in µg/mL) from **Table 1.3**. Indicate your answer in the **Answer Sheet.** 6.0pt

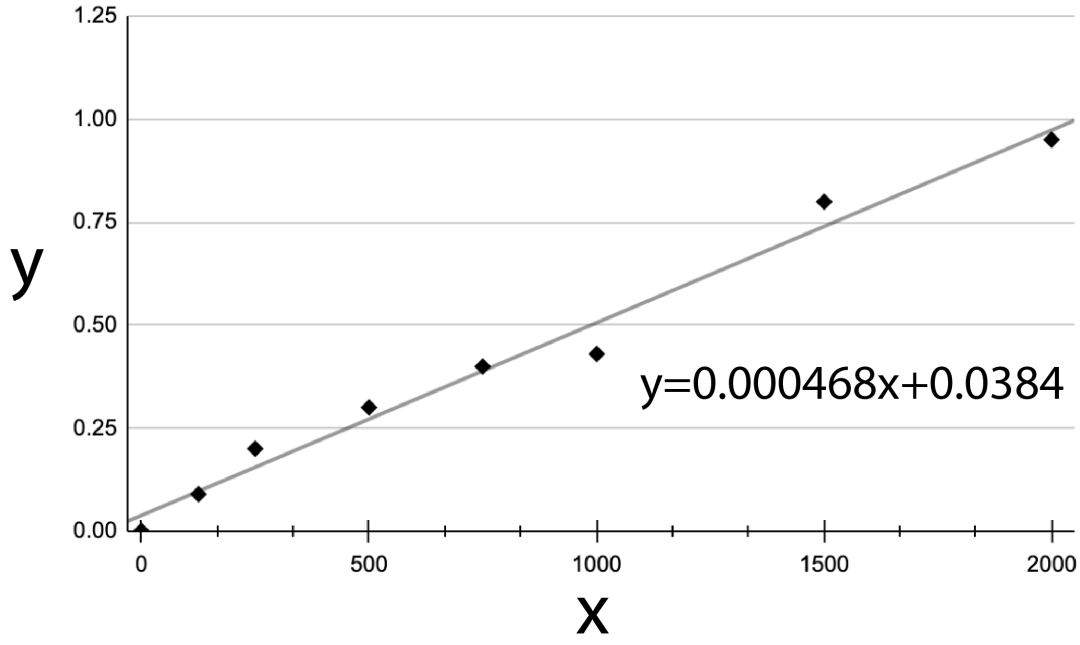


Figure 1.3. Standard curve. x = Concentration (µg/mL), **y =** Absorbance (A_{595}).

Sample #	**Absorbance** (triplicate, A_{595})		
Tube I (Sample 1)	0.674	0.742	0.693
Tube J (Sample 2)	0.313	0.376	0.298

Table 1.3: Absorbances of Sample 1 **and** Sample 2**.**

Question 1.4. Ion-exchange chromatography allows you to separate ions and polar molecules based on their charges. In this experiment, both samples were purified using ion exchange chromatography. Sample 1 was purified using cation-exchange chromatography with a negatively charged immobile phase, while Sample 2 was purified by anion-exchange chromatography with a positively charged immobile phase. The bound molecules eluted and collected by changing the pH of the column. At the beginning of elution, pH of the column resembled the physiological pH of the blood and gradually increased. According to your results from **Table1.3**, what can be concluded about SUB1? Indicate your answer in the **Answer sheet**. 3.0pt

a) No statement about the net charge can be made from the measurements

b) SUB1 protein has a positive net charge at physiological pH.

c) SUB1 protein has a negative net charge at physiological pH.

d) SUB1 protein has no net charge at physiological pH.

Part II: Identifying compounds based on their titration curves

In the second part of the exam, you will perform compound titration for Compound 1 and Compound 2 by using sodium hydroxide (NaOH). For Compound 3 and Compound 4, you will be provided with the ready titration charts (Figures 2.4 and 2.5).

Note that all four compounds are fully protonated before the experiment.

Protocol:

Very important: Wear a lab coat, gloves, and safety goggles throughout the **ENTIRE** exam. **Note**: wearing safety goggles is optional if you already wear glasses for vision.

A. Turn on the magnetic stirrer by pressing the "POWER" button.

B. Place a magnetic stirring bar inside a clean 100 mL beaker labeled "COMPOUND 1".

C. Using a 25 mL serological pipette and the pipette aid (Stripettor Ultra), take 20 mL of the "COMPOUND 1" solution and put the solution into the beaker from step **B.**

D. Place the beaker with Compound 1 and the magnetic stirring bar on the magnetic stirrer.

E. Take the pH meter probe out of the "ELECTRODE STORAGE" solution. Rinse it with water and dispense the water into a beaker labeled "LIQUID WASTE" **(Figure 2.1)**. Then, gently wipe the probe with Kim-wipe tissue paper to remove excess water.

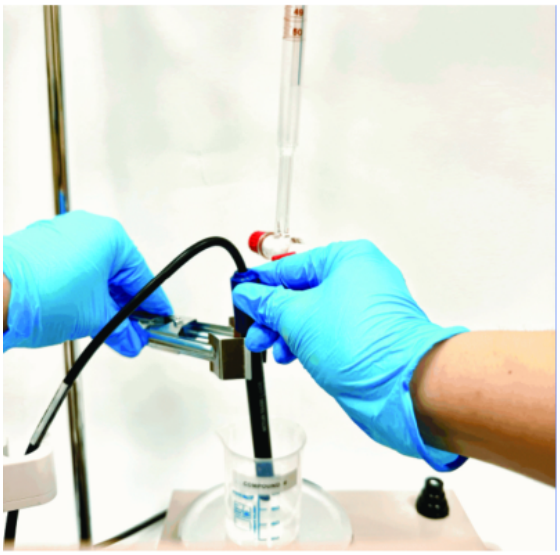
Figure 2.1. Rinsing the pH meter probe.

F. Next, submerge the pH meter probe into the beaker from step **D**. **Important: The probe should be dipped far into the solution but not touch the magnetic bar or beaker.**

G. Carefully clamp the pH meter probe to the retort stand as shown in **Figure 2.2 (Make sure that the stirring bar will not hit the probe while stirring)**. **Note**: the pH meter is already calibrated and ready to use.

H. Adjust the stirring speed by turning the rotation regulator clockwise carefully.

A



B

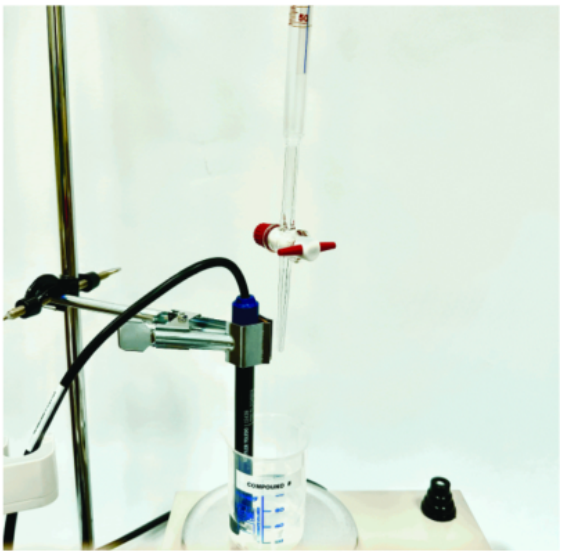


Figure 2.2. The pH meter probe on the retort stand.

I. From the pH meter screen **(Figure 2.3)** note the starting pH of the solution by pressing the **button Read.** Record your answer in the **Answer Sheet.**

Question 2.1. Write down the starting pH in the table in the **Answer Sheet**.	1.6pt

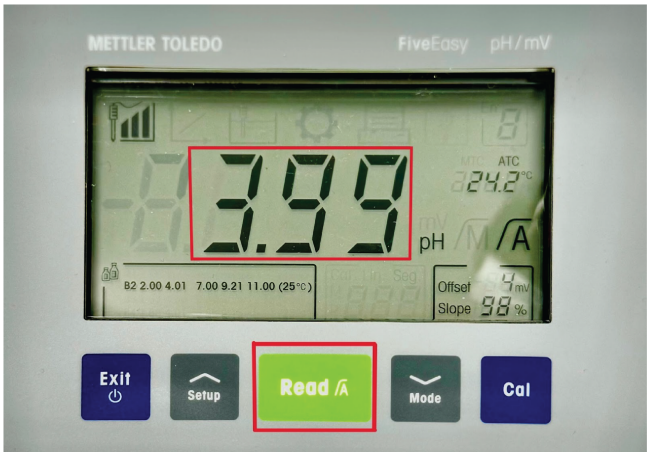


Figure 2.3. The screen of the pH meter.

J. Slowly and carefully dispense 1.00 mL of NaOH solution at a time from the burette into the solution and press the **button Read**. Record the pH (rounded to two decimal places) of the solution after each measurement in the **Question 2.2** table of your **Answer Sheet.** If you accidentally add more than the necessary amount of NaOH, adjust the volume to the nearest integer for the next measurement. **Note: You have 50 mL of NaOH solution ready in the burette for the titration of two compounds.**

	Question 2.2. Measure and write down the pH values (rounded to two decimal places) in the titration table in the **Answer Sheet**	32.0pt

K. Repeat step J until 20 mL of NaOH has been added to the solution.

L. Carefully remove the pH meter probe from the retort stand clamp and rinse it with water as described in step E.

M. Carefully put the pH meter probe back into the "ELECTRODE STORAGE" solution.

N. Remove the beaker containing Compound 1 from the magnetic stirrer.

O. For Compound 2, repeat steps B-K using **the remaining NaOH solution in the burette.**

P. After measuring the pH values of both compounds, rinse the pH meter probe as described in step E.

Q. Finally, submerge the pH meter probe into the "ELECTRODE STORAGE" solution.

Note: From now on, you will not perform any experiments. You only need to answer the following theoretical questions.

The careless researcher, Daniyar, was supposed to perform titration for Compounds 3 and 4. Unfortunately, he forgot to properly label the tubes during his experiment. As a result, he currently does not know the identity of the compounds. Now, he labels the tubes as Compound A and Compound B and performs the titration. He knows that Compound 3 has two functional groups, one acidic and one basic, that can donate or accept a proton, while Compound 4 has an extra basic functional group. Based on this information, answer the following theoretical Question 2.3, Question 2.4, Question 2.5.

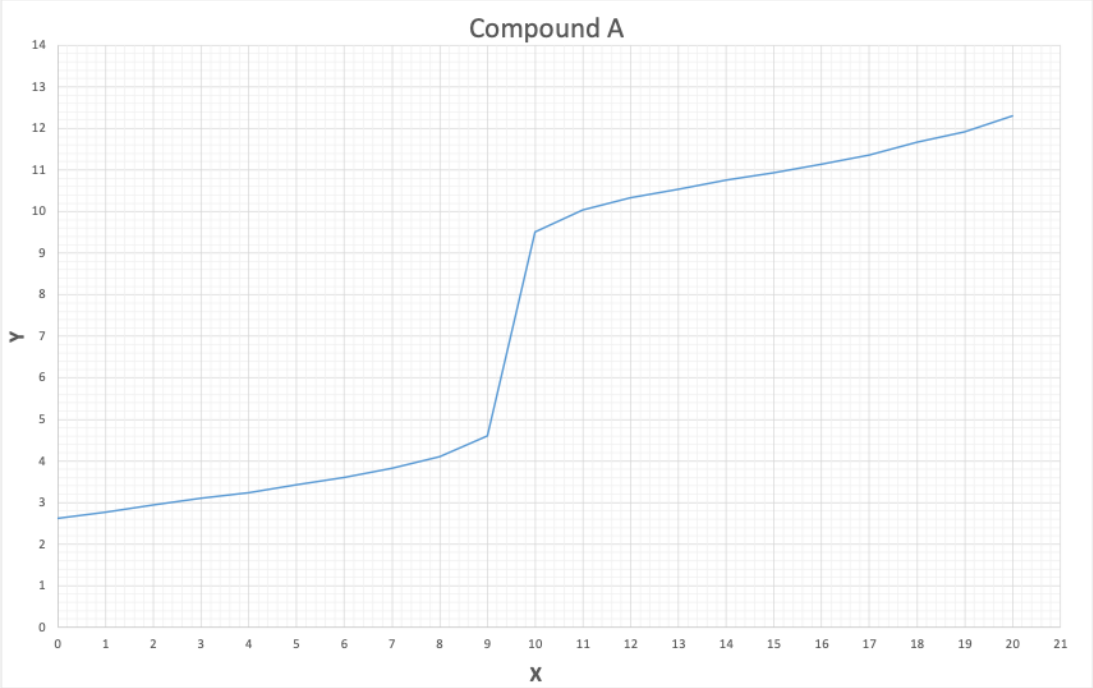


Figure 2.4. Titration chart for Compound A**. X** = Volume of NaOH added in mL, **Y** = pH.

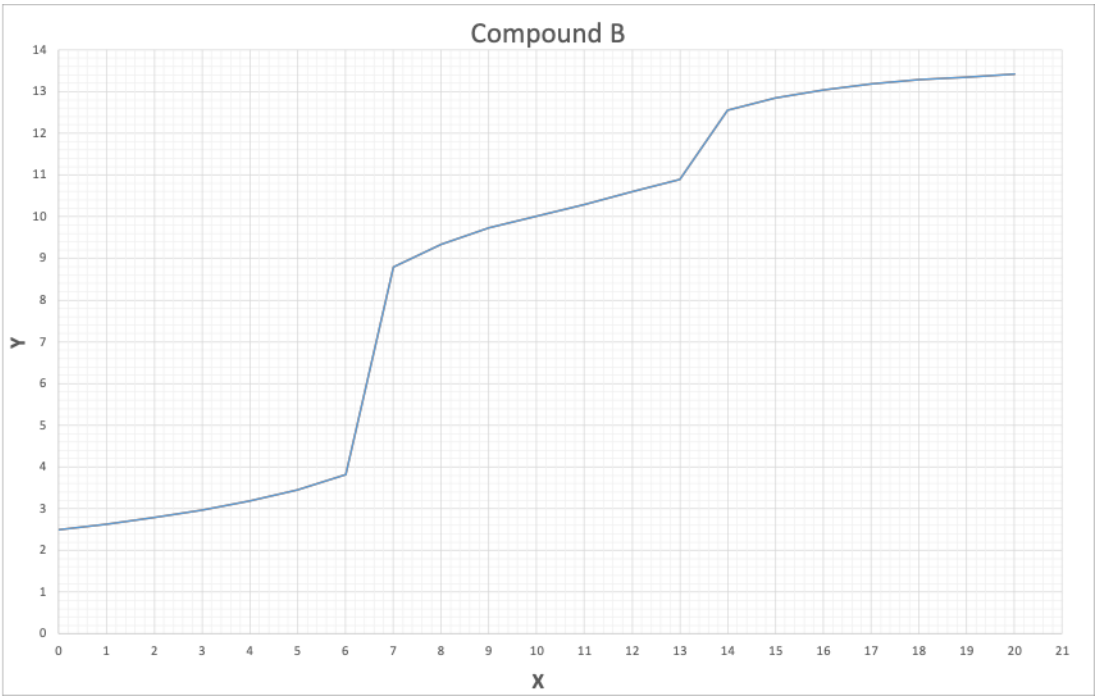


Figure 2.5. Titration chart for Compound B**. X** = Volume of NaOH added in mL, **Y** = pH.

Question 2.3. Based on the information given above, match the identity of compounds A and B with compounds 3 and 4 in the **Answer Sheet.** 3.4pt

Question 2.4. Based on the curves provided in the Figures 2.4 and 2.5, extrapolate the pK_a values for each functional group. Write down the pK_a values (rounded to one decimal place) in the **Answer Sheet.** 10.0pt

Question 2.5. The normal physiological pH level of human blood is around 7.4. Using the pK_a values of each of these compounds (from Question 2.4), predict and indicate the net charge of each of these compounds in blood in the **Answer Sheet**. Use the following information: 3.0pt

1) The acidic group has no net charge when protonated and a negative (-1) charge when deprotonated.
2) Both basic groups have a positive (+1) charge when protonated and no net charge when deprotonated.

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35th International Biology Olympiad

Practical Exam: Biochemistry

Answer Sheet

Important!

To ensure that the numbers you write in this answer sheet are clearly distinguishable to the Scientific Committee members of the IBO2024, please write them as you normally would in the following box.

0	1	2	3	4	5	6	7	8	9

Part I: Quantifying protein purification efficiency

1.1 (35 pt)
Question 1.1. After finishing step F, cover the plate with aluminum foil. Raise your Spectrophotometer measurement card (Spectrophotometer image) and pass the prepared 96-well plate to the assistant for measuring absorbance (35.0 pt).

1.2 (6.0 pt)
Question 1.2. Calculate and write down the final protein concentrations (rounded to the nearest integer in micrograms/mL) in the blank spaces in the following box (6.0 pt)

Tube #	The volume of dH_2O in µL	Volume in µL and (Source of BSA)	Final protein concentration in µg/mL
1	-	25 (stock)	2000
2	13	39 (stock)	
3	25	25 (stock)	
4	25	25 (Tube 2)	
5	25	25 (Tube 3)	
6	25	25 (Tube 5)	
7	25	25 (Tube 6)	
8	25	-	0

1.3 (6.0 pt)
Question 1.3. Calculate the average absorbances of Sample 1 and Sample 2 (rounded to three decimal places) and the protein concentrations (rounded to two decimal places) and write them down in the blank spaces in the following box (6.0 pt).

Sample #	**Average absorbance**	**Protein concentration (µg/mL)**
Tube I (Sample 1)		
Tube J (Sample 2)		

1.4 (3.0 pt)
Question 1.4. Circle the correct answer (3.0 pt).

a) No statement about the net charge can be made from the measurements.

b) SUB1 protein has a positive net charge at physiological pH.

c) SUB1 protein has a negative net charge at physiological pH.

d) SUB1 protein has no net charge at physiological pH.

Part II: Identifying compounds based on their titration curves

2.1 (1.6 pt)
Question 2.1. Write down the starting pH values in the blank spaces in the following box (1.6 pt).

	Compound 1	Compound 2
Starting pH		

2.2 (32.0 pt)
Question 2.2. Measure and write down the pH values (using two decimal places) in the blank spaces in the titration table (32.0 pt).

	pH measured	
Volume of NaOH added in mL	Compound 1	Compound 2
1.00		
2.00		
3.00		
4.00		
5.00		
6.00		
7.00		
8.00		
9.00		
10.00		
11.00		
12.00		
13.00		
14.00		
15.00		
16.00		
17.00		
18.00		
19.00		
20.00		

2.3 (3.4 pt)
Question 2.3. Mark the identity of the compounds with "√" (3.4 pt).

	Compound A	Compound B
Compound 3		
Compound 4		

2.4 (10.0 pt)
Question 2.4. Write down the pK_a values (using one decimal place) in the blank spaces in the table below. Note that the Compound 3 does not have basic group 2 (10.0 pt).

	Acidic group (pK_a)	Basic group 1 (pK_a)	Basic group 2 (pK_a)
Compound 3			X
Compound 4			

2.5 (3.0 pt)
Question 2.5. Using the pK_a values of each of these compounds, predict the net charge of each of these compounds in the human blood (3.0 pt).

	Net charge
Compound 3	
Compound 4	

Student name: _____	Student code: _____

35th International Biology Olympiad

7th-14th July 2024 Astana, Kazakhstan

Practical Exam: Biochemistry

Answer Key

Total points: 100
Duration: 90 min

Part I: Quantifying protein purification efficiency

Question 1.1 35 points. (-5 points for turned plates)

Point distribution for students' prepared samples (from the Excell calculator)
Correct absorbance values must be determined empirically by the organizers

Task	In total 35 points	Detailed point distribution
Average Absorbance values	20 points	If triplicate: 2.5 points +/- 10%, 1.25 point +/- 20% If duplicate: 1 points +/- 10%, 0.5 point +/- 20% If single: 0.5 points +/- 10%, 0.25 point +/- 20%
R square	5 points total	If R square is >0,95 → 5 points
		If R square is > 0.90 → 3 points
		If R square is > 0.85 → 1 points
		For any other value → 0 points
Unknown samples	5 points each	If the answer is in the range +/- 10% → 5 points
		If the answer is in the range +/- 20% → 3 points

Question 1.2. Calculate and write down the final protein concentrations in tubes **(1 point for each, a total of 6 points)**

Tube #	The volume of dH2O in µL	Volume in µL and (Source of BSA)	Final protein concentration in µg/mL
1	-	25 (stock)	2000
2	13	39 (stock)	**1500**
3	25	25 (stock)	**1000**
4	25	25 (Tube 2)	**750**
5	25	25 (Tube 3)	**500**
6	25	25 (Tube 5)	**250**
7	25	25 (Tube 6)	**125**
8	25	-	0

Question 1.3. Calculate the average absorbance (rounded to three decimal places) and the protein concentrations (rounded to two decimal places) **(in total 6 points)**.

Sample	Absorbance			Average Absorbance **(1 point each)**	Protein Concentration (µg/ml) **(2 points each)**
(Sample 1)	0.674	0.742	0.693	**0.703**	**1420.09** **(1420.09) → 2 points**
(Sample 2)	0.313	0.376	0.298	**0.329**	**620.94** **(620.94) → 2 points**

Question 1.4. Select the correct answer **(3 points)**

a) SUB1 protein has a high molecular weight.

b) SUB1 protein has a positive net charge at physiological pH.

c) SUB1 protein has a negative net charge at physiological pH.

d) SUB1 protein has no net charge at physiological pH.

Part II: Identifying compounds based on their titration curve

Question 2.1. The starting pH **(0.8 points for each titration)**

	Unknown Compound 1	Unknown Compound 2
Starting pH	**Any answer**	**Any answer**

Question 2.2. Titration table **(32 points total)**

1. **0,5 points per measurement if the answer is in the given range**
2. **For compound 1: calculate the biggest change for consequent measurements between 8.00 mL and 13.00 mL NaOH measurements and if there is a change in pH larger than 4.0 → 4 points.**
3. **For compound 2:**
    a. **We will calculate the biggest change for consequent measurements between 4.00 mL and 9.00 mL NaOH measurements and if there is a change in pH larger than 3.4 → 4 points**
    b. **We will calculate the biggest change for consequent measurements between 11.00 mL and 16.00 mL NaOH measurements and if there is a change in pH larger than 1.0 → 4 points**

	pH measured	
NaOH added (mL)	Compound 1	Compound 2
1	1,66 (+/-0,15)	1,72 (+/-0,15)
2	1,81 (+/-0,15)	1,88 (+/-0,15)
3	1,96 (+/-0,15)	2,04 (+/-0,15)
4	2,12 (+/-0,15)	2,25 (+/-0,15)
5	2,27 (+/-0,15)	2,49 (+/-0,25)
6	2,44 (+/-0,15)	2,88 (+/-0,25)
7	2,6 (+/-0,15)	6,92 (+/-0,25)
8	2,8 (+/-0,15)	8,37 (+/-0,25)
9	3,06 (+/-0,25)	8,76 (+/-0,15)
10	3,54 (+/-0,25)	9,03 (+/-0,15)
11	8,24 (+/-0,25)	9,31 (+/-0,15)
12	8,98 (+/-0,25)	9,6 (+/-0,25)
13	9,3 (+/-0,15)	10,06 (+/-0,25)
14	9,51 (+/-0,15)	11,34 (+/-0,25)
15	9,71 (+/-0,15)	11,84 (+/-0,25)
16	9,88 (+/-0,15)	12,05 (+/-0,15)
17	10,07 (+/-0,15)	12,18 (+/-0,15)
18	10,27 (+/-0,15)	12,28 (+/-0,15)
19	10,52 (+/-0,15)	12,35 (+/-0,15)
20	10,93 (+/-0,15)	12,41 (+/-0,15)

For questions 2.3~2.5, note that we changed values in the provided charts by increasing all the values by 1.00.

Question 2.3. Mark with an X the identity of the compounds **(1.7 points for each right answer, a total of 3.4 points: if the student indicated both unknown compounds' identities are the same (i.e. both unknown compounds are Drug-a), then no points)**.

	Compound A	Compound B
Compound 3	**X**	
Compound 4		**X**

Question 2.4. Write down the pKa values in the table below **(In total 10 points):**

	Acidic group (pKa)	Basic group 1(pKa)	Basic group 2 (pKa)
Compound 3	**3.2~3.4**	**10.8~11.1**	**X**
Compound 4	**2.8~3.1**	**9.8~10.2**	**13.1~13.2**

Question 2.5. Using the pKa values of each of these compounds, please predict the net charge of each of these compounds in the blood **(1,5 points for each right answer, a total of 3 points)**

	Net charge
Compound 3	**0**
Compound 4	**+1**