Workplace number _____ Name _____

58-th INTENATIONAL MENDELEEV OLYMPIAD

PRACTICAL EXAMINATION

Shenzhen – 2024

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General Directions

- **Safety rules:** follow the conventional rules of carrying out chemical experiments; when in the lab, you are required to wear the lab coat and safety goggles (or your prescribed glasses); no eating or drinking in the lab.
- **Violating safety rules**: you get one warning, offend again: you are disqualified.
- **Time:** the total duration of the experimental exam is 5 h. **You will have 15 min to read the text. You are not allowed to start working during this period** (you can look at the sampling pipette and other equipment).
- The lab is equipped with clocks. Once the STOP command is given, you must immediately stop working and deliver all sheets.
- **Besides chemicals, labware and equipment, use only calculator and pen.**
- Approach your lab assistant, if you got **questions** on lab safety or you need a toilet break.
- Dispose **liquid waste** in the plastic container labelled "**Liquid waste**". You will find it at your table.
- Write down **answers only in the designated places**; answers written elsewhere will not be marked. Give calculations where appropriate.
- **You can re-use glassware.** Wash these carefully.
- The reagents given are limited. Spilled or totally used solution will be substituted with penalty.
- Using **burettes**: you are given burettes with open stopcocks. Close it before use. If there is a bubble left in the tip when filling the burette, position it vertically and carefully shake. If the bubble is still there, seek for help from your lab assistant. The burette may leak. If so, fasten the plastic stopcock adaptor. Always place a beaker under the burette.
- You can use the backside of the exam sheets as draft paper.
- **Keep your workspace in order.** There are tissue rolls at each table. Besides, there are rolls of paper towels in each lab.
- If you have broken an item, approach your lab assistant, who will help you to fix the case and provide you with a replacement item.
- You will be using a sampling pipette with the range of volumes from 100 up to 1000 μL. Adjust the required volume by turning the ring. Pressing the piston to the first stop allows filling the tip with the adjusted volume, pressing the piston to the final stop position releases the entire volume. Be sure not to suck the liquid inside the pipette itself, which may destroy it. Use a separate tip for each individual solution**.**
- You are expected to use a **3-valve bulb** to fill glass pipettes. It is completely prohibited to fill the pipettes by mouth. The pipette filler has three valves (glass balls): A for air draining when pressing the bulb, S for sucking a solution into the pipette, E for draining the pipette. The valves are difficult to press; please apply power!

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Spirulina platensis – superfood


Spirulina, very popular in China and a number of neighboring countries, is a blue-green microalgae with a unique composition. It contains up to 70% of valuable protein, which is easily absorbed by the body, as well as many vitamins, microelements and other useful substances. Spirulina is rich in unsaturated fatty acids, such as linoleic and linolenic acids, which play a key role in the status of human cardiovascular system. Spirulina is rich in polysaccharides and photosynthetic pigments, including phycocyanin, chlorophyll and phycoerythrin, which have a positive effect on metabolism and functioning of the digestive system. Thus, spirulina is a multifunctional means of maintaining health and improving functioning of various body systems.

Part A. Conformations of phycocyanin

Spirulina is rich in protein phycocyanin, which is beneficial to humans as it promotes the production of red blood cells, white blood cells and platelets, as well as the growth and differentiation of stem cells. Although proteins are composed of the same amino acids, some proteins are colored, and some are not. Phycocyanin has a bright color due to a chromophore called phycocyanobilin.

Reagents:

Reagent	Label	Placed in
Phycocyanin solution	Phycocyanin	10 mL vial
Buffer solution (pH 7.0)	Buffer	10 mL vial
Water	H_2O	Wash bottle
Sodium chloride solution	NaCl	Eppendorf tube
Guanidinium chloride solution	Guanidine	Eppendorf tube
Sodium lauryl sulfate solution[1]	SDS	Eppendorf tube
Urea solution, 8 M	Urea 8 M	10 mL vial
Sodium hydroxide solution, 1 M	NaOH 1 M	Eppendorf tube
Hydrochloric acid solution, 1 M	HCl 1 M	Eppendorf tube
Acetone	Acetone	Eppendorf tube
Ethanol	Ethanol	Eppendorf tube
Ammonium sulfate solution	$(NH_4)_2SO_4$	Eppendorf tube

Equipment: cuvettes, photometer, sampling pipette, tips, Eppendorf tubes with rack.

[1] There may be precipitate in the solution, which does not interfere the experiment. One can reduce the amount of the precipitate by heating and shaking the solution.

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When phycocyanin folds around phycocyanobilin, a bright blue color appears, indicating whether the protein is folded or unfolded. Various substances that change the conformation can be used to test the conformational state of the protein.

In a 10 mL vial you are given a solution of phycocyanin in a buffer solution. Using the sampling pipette, pour 100 µl of phycocyanin solution into 11 Eppendorf tubes. Then add 900 µl of analyzed reagents to each Eppendorf tube. Close the tube and shake it several times. Equilibrium in the systems is established within 5 minutes.

Determine how the color of the solution has changed using the following notation:

"**B**" is for deep blue, take the color of phycocyanin in the buffer solution as a reference;
"**L**" is for light blue;
"**P**" is for purple;
"**O**" for colorless;
"**X**" for any other coloring.

A1. Place the Eppendorf tubes according to their numbers in the Sheet 5. Record your observations according to the notations provided. Get your lab assistant's signature.

A2. Which reagent is most efficient in unfolding the protein according to the analysis performed?

A3. Changes in what factors do lead to protein denaturation?

- □ Temperature
- □ Ionic strength
- □ Medium acidity
- □ Protein concentration

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Example	Write down the color code: **B/L/P/O/X**	**1. Buffer**		**2. H_2O**		**3. NaCl**		**4. Guanidine**		**5. SDS**	
 Place Eppendorf tube here for photographing											
6. Urea 8 M		**7. NaOH 1 M**		**8. HCl 1 M**		**9. Acetone**		**10. Ethanol**		**11. $(NH_4)_2SO_4$**	

Lab assistant's signature _____

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Many proteins perform their functions in the form of quaternary structures, the energy of which differs from that of the linear biopolymer. Estimating the difference in energy between protein structures is not an easy task, but colored proteins can serve as a convenient model for such calculations.

To calculate the Gibbs free energy of the protein unfolding process, prepare 7 phycocyanin solutions with a total volume of 2 mL each, using different volumes of 8 M urea solution according to the table below. Using the sampling pipette, add 200 μL of phycocyanin and the corresponding volume of the urea solution to plastic cuvettes (for measuring optical density), then add the calculated volume of the buffer solution and mix well (you can do this by multiple insertion of the solution into the pipette tip). Label the cuvettes with a marker. Wait approximately 20–30 min to establish equilibrium. During this time, you can fulfil other parts of the exam set.

Cuvette number	**1**	**2**	**3**	**4**	**5**	**6**	**7**
***V*(Phycocyanin), μL**	200	200	200	200	200	200	200
***V*(Urea 8 M), μL**	0	750	900	1100	1300	1400	1800
***V*(Buffer), μL**							

A4. Calculate the concentration of urea in the numbered test tubes. Record your results in the table.

Cuvette number	**1**	**2**	**3**	**4**	**5**	**6**	**7**
***c*(Urea), M**							

After the given time, record the transmittance (*T*, %) of each solution using a monochromatic light **transmittance sensor** (photometer) located next to the computer. If the device is switched off, then allow it to warm up for about 30 s after switching on before starting measurements. To carry out measurements, open the lid and place the cuvette in the cuvette holder with the arrow pointing towards the screen. Measurements are carried out at the wavelength of 650 nm (Red(650nm) and light transmittance are displayed on the device screen). **It is important to carry out all measurements using the same device!**

The transmittance *T* is related to the absorbance (*A*) by the following relationship:

$$A = -\lg\left(\frac{T,\%}{100}\right)$$

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A5. Indicate the number of the photometer you are using. Record the measured transmittance values in the table. Calculate the absorbance values for each sample.

The number of the photometer: _____

Cuvette number	**1**	**2**	**3**	**4**	**5**	**6**	**7**
***T*, %**							
A							

Use the next formula to calculate the equilibrium constant for protein unfolding:

$$K_{\text{eq}} = \frac{[\text{unfolded}]}{[\text{folded}]} = \frac{A_{\text{f}} - A}{A - A_{\text{u}}},$$

where K_{eq} is the equilibrium constant, A_{f} is the absorbance of the folded protein (in the buffer system), A_{u} is the absorbance of the unfolded protein (at the highest urea concentration), A is the absorbance of the solution for which the constant is calculated.

A6. Calculate the equilibrium constant and Gibbs free energy (20°C) of the protein unfolding in accordance with the cuvette number.

Cuvette number	**2**	**3**	**4**	**5**	**6**
$\boldsymbol{K_{\text{eq}}}$					
$\boldsymbol{\Delta_{\text{r}}G°}$					

A7. On graph paper (next sheet), plot the dependence of $\Delta_r G^\circ$ on the urea concentration and draw the trend line by hand. Indicate the dimensions along the axes.

A8. Calculate the Gibbs free energy in the aqueous solution ($\Delta_r G^\circ_{\text{H}_2\text{O}}$) from the calibration plot, assuming the following form of the dependence:

$$\Delta_r G^\circ = \Delta_r G^\circ_{\text{H}_2\text{O}} - m \cdot c,$$

where m is a certain coefficient, c is the concentration of urea.

Write down your answer:

$\boldsymbol{\Delta_{\text{r}} G^\circ_{\text{H}_2\text{O}}} =$

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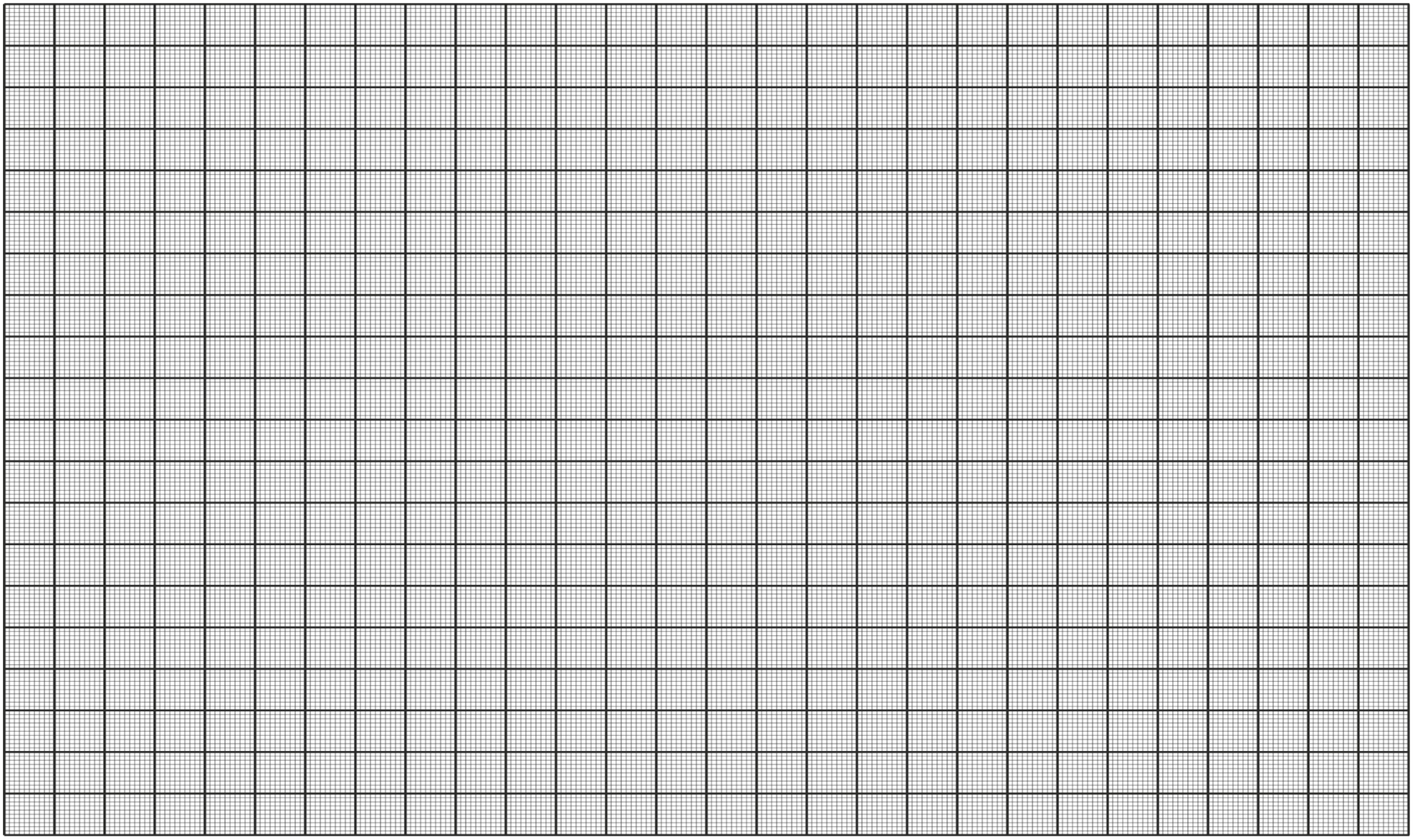

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Part B. Titrimetric determination of fatty acids using the Margosches method

In addition to the protein, spirulina contains unsaturated fatty acids. Determination of the iodine value is behind one of the main ways to find the unsaturation of fats. The iodine value expresses the mass of I_2 (in grams) required to completely saturate the fatty acids contained in 100 g of fat. The Margosches method allows determining the iodine value, based on the oxidation of a double bond with iodine, followed by titration of the unreacted iodine. You will have to determine the mass of the oil sample given to you based on the titration results and the iodine value.

Reagents:

Reagent	Label	Placed in
Sodium thiosulfate solution, 0.09621 M	$Na_2S_2O_3$	250 mL bottle
Margosches solution (ethanolic solution of iodine, 0.1 M)	I_2	100 mL bottle
Starch solution, 1%	Indicator (starch)	30 mL drop-bottle
Alcohol mixture ethanol-chloroform (10:1 vol.)	C_2H_5OH $CHCl_3$ (10:1)	250 mL bottle

Equipment: 100 mL volumetric flask with a sample of oil (1 pc.), 25 mL burette (1 pc.), small glass funnel for the burette (1 pc.), bulb with three valves (1 pc.), 100 mL graduated cylinder (1 pc.), 10 mL graduated pipette (3 pcs.), 250 mL titration flask with ground stopper (3 pcs.), 50 mL beaker (3 pcs.).

Pour the standard sodium thiosulfate solution into the burette. Using the beaker, add 40 mL of the mixture of ethanol and chloroform (10:1) (hereinafter referred to as the "alcohol mixture") in the volumetric flask with the sample of oil and mix vigorously. Do not turn the flask upside down. Make sure the sample has dissolved. Bring the solution to the mark with the alcohol mixture, mix vigorously again, turning the flask upside down.

Reference experiment. To carry out a reference experiment, add 10.00 mL of the mixture of ethyl alcohol and chloroform (10:1) and 5.00 mL of Margosches solution into a 250 mL conical flask using a pipette. Then add 40 mL of distilled water, stopper, shake, **open the stopper slightly to release pressure in the flask** and leave for 5 min. Then carefully shake the flask with the closed stopper 2–3 times, holding the stopper, and **open the stopper slightly to release the pressure in the flask**. Stir the contents of the flask vigorously for 3 min, periodically releasing the pressure.

Stirring vigorously, titrate the resulting mixture with the sodium thiosulfate solution until a light yellow color appears (**make sure there is no dark precipitate left at the bottom of the flask**), then add 3–4 drops of starch and titrate until the blue color disappears.

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B1. Record the volume of sodium thiosulfate spent in the reference experiment (V_{ref}):

Titration No	1	2	3			
The initial volume, mL						
The final volume, mL						
The volume spent for the titration, mL						

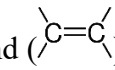
The accepted volume, V_{ref}: _____ mL

Determination of the iodine value of the oil mixture. The procedure described for the reference experiment must be carried out for the solution of oil in the alcohol mixture. Transfer a 10.00 mL aliquot of the oil solution from the volumetric flask into a titration flask. Then add 5.00 mL of Margosches solution to the analyzed sample using a pipette. Add 40 mL of distilled water, stopper the flask, shake, **open the stopper slightly to release the pressure in the flask** and put the flask aside for 5 min. Then gently shake the flask with the stopper closed **and open the stopper slightly to release the pressure in the flask**. Stir the contents of the flask vigorously for 2–3 min, releasing the pressure from time to time. Stirring vigorously, titrate the resulting mixture with the solution of sodium thiosulfate until the color changes from brown to light yellow. Then add the indicator (1% starch solution) and continue titration until the blue color disappears.

B2. Record the volume of sodium thiosulfate spent on titrating the sample with oil (V):

Titration No	1	2	3			
The initial volume, mL						
The final volume, mL						
The volume spent for the titration, mL						

The accepted volume, V: _____ mL

B3. Write the equation for the reaction of an unsaturated compound () with the Margosches reagent in the presence of water. Note that vicinal diiodides are unstable and are not formed in this reaction.

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B4. Derive a formula relating the iodine value of the oil given to you, the mass of the oil in your volumetric flask m (mg), the volume of the solution in the 100.0 mL volumetric flask, 10.00 mL volume of the aliquot, and the volumes (mL) obtained during titrations (V_{ref} for the reference, V for the actual determination), the concentration of thiosulfate $c(Na_2S_2O_3)$, and the mass of the iodine equivalent (M = 126.9 g/mol).

Your work:
Iodine value =

B5. Calculate the mass of oil in the sample given to you if its iodine value is **86**.

Calculations: **The oil content in the volumetric flask:_____ mg**

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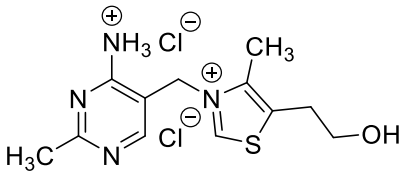
Part C. Identification of vitamins

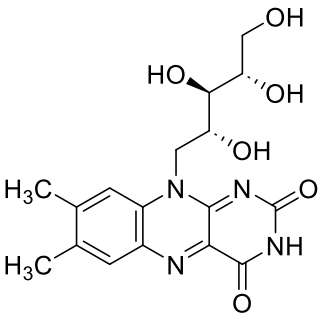
Spirulina is a unique microalgae containing vitamins and microelements necessary for humans. Spirulina contains vitamins such as A, B_1, B_2, B_3, B_6, B_9, C, D, and E. These vitamins play an important role in maintaining the health of our skin, hair, nails, immune system, and general health of the body.

The table below lists some of the vitamins that spirulina may contain.



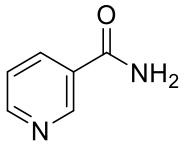
Thiamine hydrochloride
(vitamin B_1)

($C_{12}H_{17}ClN_4OS$)



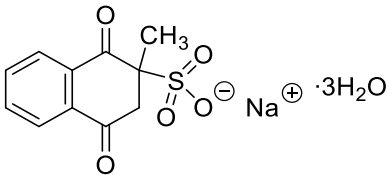
Riboflavin (vitamin B_2)

($C_{17}H_{20}N_4O_6$)



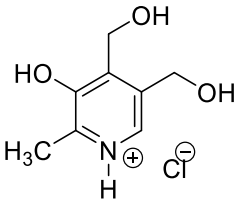
Nicotinamide
(vitamin B_3)

($C_6H_6N_2O$)



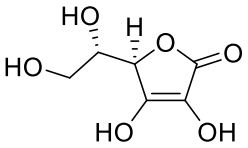
Vikasol
(a vitamin K_3 analog)

($C_{11}H_9NaO_5S$)



Pyridoxine hydrochloride
(vitamin B_6)

($C_8H_{12}NO_3Cl$)



Ascorbic acid (vitamin C)

($C_6H_8O_6$)

Reagents:

Reagent	Label	Placed in
Thiamine hydrochloride	**1–8**	Eppendorf tube
Riboflavin		Eppendorf tube
Nicotinamide		Eppendorf tube
Vikasol		Eppendorf tube
Pyridoxine hydrochloride		Eppendorf tube
Ascorbic acid		Eppendorf tube
Potassium iodide solution		Eppendorf tube
Phosphate buffer (NaH_2PO_4/Na_2HPO_4)		Eppendorf tube
Sodium hydroxide solution, 0.1 M	NaOH 0.1 M	Eppendorf tube
Sodium nitrite solution, 1 M	$NaNO_2$ 1 M	Eppendorf tube
Iron(III) chloride solution, 0.05 M	$FeCl_3$ 0.05 M	Eppendorf tube

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Sulfanilic acid solution†, 0.06 M	Sulfanilic acid 0.06 M	Eppendorf tube
Silver nitrate solution, 0.5 M	$AgNO_3$ 0.5 M	Eppendorf tube
Indicator paper for determination of pH		

Equipment: 96-well plate, filter paper, sampling pipette, replaceable tips.

You have at your disposal a set of the following reagents: NaOH, $NaNO_2$, $FeCl_3$, sulfanilic acid, $AgNO_3$. Using these solutions individually or mixing them and obtaining new reagents, you can identify vitamins and other substances offered to you. You are given solutions of vitamins: thiamine hydrochloride, riboflavin, nicotinamide, vikasol, pyridoxine hydrochloride, ascorbic acid (see the formulas in the table above) in Eppendorf tubes labelled **1**–**8**. In addition, the KI solution and phosphate buffer are among substances to be identified.

Identification of vitamins is based on the ability of substances to lead to colored products, as well as to the precipitate formation of and (or) gas release when treated with appropriate reagents. Also use the provided reagents to create the pH value necessary for the reaction. Use universal indicator paper to monitor pH.

To successfully complete the experiment, pay attention to the following recommendations:

• carry out reactions in the 96-well plate;

• do not pour too much solution into the cells. It is enough to add 100 μL of the analyzed solution to carry out the reaction.

When performing identification, consider the following facts:

• Riboflavin in a neutral or slightly acidic solution (pH 6.5−7.2) forms a colored compound when reacting with $AgNO_3$.

• Vitamin B_6 and nicotinamide form colored compounds with $FeCl_3$.

• Diazophenylsulfonic acid is formed as a result of the reaction of sulfanilic acid with an acidified solution of sodium nitrite.

• Thiamine reacts with diazonium salts in an alkaline environment to form red-orange colored triazenes. Pyridoxine is also capable of reacting with azo coupling in an alkaline medium to form red-orange colored products.

• In an alkaline environment (pH ≥ 11), the sulfonate group is removed from the vikasol molecule, and 2-methyl-1,4-naphthoquinone precipitates.

†4-Aminobenzenesulfonic acid ($C_6H_7NO_3S$)

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The table for recording your observations (not graded):

		The reagents added						
No of the test tube with the unknown substances	**1**							
	2							
	3							
	4							
	5							
	6							
	7							
	8							

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C1. Determine the contents of the test tubes. Write your answers in the table.

The solution analyzed	**B_1**	**B_2**	**B_3**	**B_6**	**K_3**	**C**	**KI**	**NaH_2PO_4 +Na_2HPO_4**
The test tube No								

C2. Write the equation for the reaction of pyridoxine hydrochloride with sodium hydroxide. Use structural formulas for organic compounds.

C3. Write the equation for the reaction of pyridoxine hydrochloride with diazophenylsulfonic acid in an alkaline medium. Use structural formulas for organic compounds.

C4. Write the equation for the reaction of ascorbic acid with iron(III) chloride. Use the molecular formulas of the compounds.

C5. Write the equation for the reaction of ascorbic acid with sodium nitrite in an acidic medium. Use the molecular formulas of the compounds.

C6. Write the equation for the reaction of potassium iodide with sodium nitrite in an acidic medium.

C7. Write the equation for the reaction of iron(III) chloride with the phosphate buffer.

C8. Write the equation for the reaction of iron(III) chloride with potassium iodide.