Equipment

Equipment	Quantity
Pour deux participants	
Hot plate	1
600 mL beaker	1
Test tube holder	1
Marker	1
Graduated cylinder (5 or 10 mL)	1
1000 mL beaker	1
Par participant	
Burette stand with clamp	1
Burette	1
Small funnel	1
Beaker (50 or 100 mL)	3
Graduated cylinder (25 or 50 mL)	1
Graduated pipette (5 mL or 10 mL)	2
Erlenmeyer flask (250 mL)	2
Watch glass	1
Pasteur pipette	11
Glass test tube	12
Beaker (50 or 100 mL)	1
Three-way pipette bulb	1
Test tube rack	1
Glass stirring rod	1
Beaker (250 mL)	1

Versatile Hydrolysis: A Kaleidoscope of Chemical Transformations

Hydrolysis is an important chemical process widely used in science and technology. For centuries, hydrolysis reactions have been employed in various fields – from the production of foodstuffs to the development of pharmaceuticals. All tasks of the Experimental Exam are related to this process; you will therefore explore different applications of hydrolysis and perform experiments involving both qualitative and quantitative analyses.

The most popular method for obtaining glucose is the hydrolysis of starch. The enzymes used in this process perform distinct functions: thermostable **α-amylase** fragments the starch, and **glucoamylase** converts the resulting short fragments into glucose. In the first task, you must carry out an enzymatic hydrolysis of starch and prove the formation of a reducing sugar. Then, using the molybdenum-blue reaction in the presence of reducing sugars, you will quantitatively determine the concentration of the glucose solution.

Titrimetry is one of the most widespread methods for the quantitative determination of substances in solution. In the second task, you will determine the glucose content via cerimetric titration. However, direct titration is not applicable to all compounds. For example, in quality control of pharmaceuticals such as paracetamol, its hydrolysis is performed first. In the third task, you are asked to determine the amount of paracetamol in the provided tablet.

A similar approach is characteristic of analyses of other organic compounds (including drugs): hydrolysis is used to remove specific functional groups, enabling effective qualitative reactions on the target substances, as you will do in the fourth task.

Each task has strict time constraints, so plan and design your experiment carefully.

Task 1. Hydrolysis of Starch and Colorimetric Determination of Glucose Concentration (16 points)

Reagent	Volume/Mass	Container	Label
For each participant			
Glucoamylase	100 μL	Eppendorf tube	Glucoamylase
Thermostable α-amylase	100 μL	Eppendorf tube	α-amylase thermostable
Starch	0.1–0.2 g	Test tube	Starch
Iodine (solution)	1 mL	Eppendorf tube	Iodine
Glucose (solution)	12 mL	Glass vial with stopper	Task # 1 Glucose #XXX
Common reagents			
Acetate buffer 0.1 M, pH 4.8		50 or 100 mL beaker	Acetate buffer pH 4.8
$(NH_4)_6Mo_7O_{24}$ 7.5%		50 or 100 mL beaker	$(NH_4)_6Mo_7O_{24}$ 7.5%
KH_2PO_4 0.2 M		50 or 100 mL beaker	KH_2PO_4 0.2 M

Equipment	Quantity
Per participant	
25 mL volumetric flask	1
On the common bench	
50 or 100 mL beaker (Acetate buffer pH 4.8/$(NH_4)_6Mo_7O_{24}$ 7.5%/ KH_2PO_4 0.2 M)	6
Graduated pipettes of various volumes for common solutions	6
Thermometer	1
Hot plate	1
600 mL beaker	1
Test tube holder	1
Heat-protective gloves	Choose your size

In this task, strict adherence to all time intervals and temperatures is required. Failure to comply with the experimental conditions will result in an incorrect outcome!

Procedure

Take the glass test tube containing the starch sample (**Starch**), remove the cap, add 12 mL of distilled water, and shake the suspension. Using a plastic pipette, transfer one drop of the suspension onto a watch glass and add one drop of iodine solution.

1-1. Indicate the result of the iodine test No. 1 on the Answer Sheet by circling **Y** if positive or **N** if negative (1 point).

Transfer quantitatively the entire provided volume of **thermostable α-amylase** to the test tube with the starch suspension and secure the tube in the holder. Place the tube into the boiling water bath for 15 min, mixing gently with a glass rod every 5 min. Fill the 250 mL beaker with cold tap water. After 15 min, remove the test tube (**using heat protective gloves – do not touch the hot plate!**), cool it in the cold-water bath to room temperature, then transfer its contents quantitatively into a 25 mL volumetric flask. Bring to the mark with distilled water and mix thoroughly.
Using a plastic pipette, transfer a few drops of this solution onto a watch glass and add one drop of iodine solution.

1-2. Indiquer le résutat du test de l'iodine No. 2 dans le tableau de réponse en encerclant **Y** si c'est positive ou **N** si c'est négative (2 points).

Mix the solution left after hydrolysis in the volumetric flask thoroughly. Take a 4.00 mL aliquot and transfer it into a clean, dry test tube. Add 4.00 mL of 0.1 M sodium acetate buffer (pH 4.8) and **the provided volume** of **glucoamylase**. Label the tube and incubate it in the **common water bath** at 60°C for 30 min, mixing occasionally with a glass rod. Remove the tube with heat protective gloves and place it in the test tube rack.

1-3. Write the overall balanced equation for the hydrolysis of starch using molecular formulas of the substances (1 point).

To detect glucose in the solution and estimate its quantity, you will carry out two experiments, which can be performed in parallel.

Detection of glucose in the starch hydrolysis products. In a clean test tube, combine 5.00 mL of the 7.5% $(NH_4)_6Mo_7O_{24}$ solution, 2.50 mL of KH_2PO_4 solution, and 5.00 mL of the hydrolysis product. Heat in the boiling water bath for 20 min, then cool in a beaker filled with water (**use heat protective gloves!**).

1.4 Show the test tube to the lab assistant and get his/her signature on your Answer Sheet (2 points).

Quantitative determination. You have been given an unknown-concentration glucose solution (**Task # 1 Glucose #XXX**). In a clean test tube, combine 5.00 mL of 7.5% $(NH_4)_6Mo_7O_{24}$, 2.50 mL of KH_2PO_4, and 5.00 mL of the unknown glucose solution. Heat in a boiling water bath for 20 min, then cool in a water bath (use heat protective gloves!). On Figure 1 (see the separate sheet), a color scale of glucose standards (mg/mL) for the above test is provided. Estimate the concentration of your unknown sample using this scale.

1.5 Tick the estimated concentration of your unknown glucose solution on the Answer Sheet (10 points). Show the test tube and your chosen interval to the lab assistant and get his/her signature.

Task 2. Titrimetric Determination of Glucose Concentration (20 points)

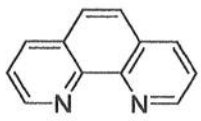
Reagent	Volume/Mass	Container	Label
Per participant			
Glucose (solution of unknown concentration)	20 mL	Glass vial with stopper	Task #2 Glucose #XXX,
Cerium(IV) sulfate (0.01044 M)	150 mL	Dark bottle with cap (250 mL)	$Ce(SO_4)_2$ 0,01044 M
H_2SO_4 15%	100 mL	Plastic bottle	H_2SO_4 15%
NaOH 2 M	30 mL	Plastic bottle	NaOH 2 M
Common reagents			
$K_3[Fe(CN)_6]$ 50 g/L (with Na_2CO_3, 20 g/L)		50 or 100 mL beaker	$K_3[Fe(CN)_6]$
Indicator ferroin		50 or 100 mL beaker	Ferroin

Equipment	Quantity
Per participant	
50 mL volumetric flask	1
On the common bench	
50 or 100 mL beaker (for $K_3[Fe(CN)_6]$ / Ferroin)	4
2 mL graduated pipette	2
5 or 10 mL graduated pipette	2

Procedure

Fill the burette with the cerium(IV) sulfate solution. In a 50.0 mL volumetric flask, add 5.00 mL of the unknown-concentration glucose solution (**Task #2 Glucose #XXX**) and 5.00 mL of the $K_3[Fe(CN)_6]$ solution (containing Na_2CO_3 to create an alkaline medium); dilute to the mark with distilled water.

Take a 10.00 mL aliquot from the volumetric flask and transfer it into a 250 mL Erlenmeyer flask. Add 2 mL of 2 M NaOH. Place the flask on the hot plate, bring to boiling (≈1 min), and boil for 60 s. Remove (**use heat protective gloves**), immediately cool under cold running water to room temperature. To the same flask, add 15 mL of 15 % H_2SO_4 and 20 mL of distilled water. Add 1.00 mL of ferroin indicator (the iron(II) complex with three molecules of 1,10-phenanthroline; see the structure below) and titrate with the cerium(IV) sulfate solution until the color changes from orange-brown to green.



1,10-Phenanthroline

Titration No.	1	2	3	4	5	6
Initial volume, cm^3						
Final volume, cm^3						
Volume consumed for titration, cm^3						
Volume accepted by you as the answer, V_{Ce}: _____ cm^3						

2.1 Record the volume of cerium sulfate used (V_{Ce}, mL). (14 points)

2.2 the ionic equations of the following reactions (use molecular formulas for organic species; 1 point each):
a) Oxidation of glucose by hexacyanoferrate(III);
b) Reaction in which cerium(IV) participates during the titration;
c) Conversion of the indicator into the blue iron(III) complex (denote phenanthroline as phen).

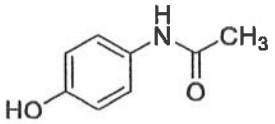
2-3. Calculate (show you work in the Answer Sheet) the concentration of the provided glucose solution, $c_{glucose}$ (mol/L). (3 points)

Task 3. Hydrolysis of Paracetamol and Determination of Its Content in a Pharmaceutical Preparation (19 points)

Reagent	Volume/Mass	Container	Label
Per participant			
Paracetamol	Portion of tablet	Conical flask (125 mL)	Paracetamol #XXX
HCl, 2 M	50 mL	Jar with cap	HCl 2 M
KBr	1 g	Eppendorf tube	KBr
$NaNO_2$, 0.0521 M	100 mL	Glass jar with lid	$NaNO_2$ for titration
Indicator paper (iodine-starch)	40 pieces	Zip-lock bag	Indicator paper

Equipment	Quantity
Per participant	
100 mL volumetric flask	1

A sample (portion of tablet) of paracetamol is placed in a 125 mL conical flask. Add 30 mL of distilled water and 30 mL of 2 M HCl to the flask. Wait until the tablet dissolves completely. Place the flask on the hot plate and boil the solution for 1 hour. Avoid vigorous boiling and excessive evaporation; reduce heating if necessary.



Paracetamol

After 1 hour, remove the conical flask from the hot plate (**use heat protective gloves!**). Cool the flask under a stream of cold water to room temperature. Quantitatively transfer the solution into a 100 mL volumetric flask, add 1 g of KBr from the Eppendorf tube (the entire amount provided), and mix thoroughly until fully dissolved. Fill to the mark with distilled water.

Fill the burette with the sodium nitrite solution.

Transfer a 10.00 mL aliquot of the prepared solution from the volumetric flask into a titration flask. Perform a preliminary titration by adding 0.5 mL portions of the $NaNO_2$ solution. After each addition, mix the solution thoroughly and, after at least 20 seconds, test completeness of the reaction using iodine–starch indicator paper. (On a dry watch glass, place pieces of indicator paper; dip a glass rod into the titrated solution and touch the paper.) Continue titration until a drop of the sample immediately produces a bright-blue coloration on the paper. Then perform the precise titration.

Titration No.	1	2	3	4	5	6
Initial volume, cm^3						
Final volume, cm^3						
Volume consumed for titration, cm^3						
Volume accepted by you as the answer, V_{NaNO2}: _____ cm^3						

3-1. Record the volume of sodium nitrite used (V_{NaNO_2}, mL) (14 points).
3-2. Write the equations (1 point each) of:
a) Hydrolysis of paracetamol in hydrochloric acid;
b) Reaction in which the hydrolysis product participates during titration;
c) Reaction behind the action of the indicator.
3-3. Calculate (show your work in the Answer Sheet) the mass of paracetamol in the sample (m, mg) (2 points).

Task 4. Analyse Qualitative des Substances Pharmaceutiques (20 points)

Reagent	Volume/ Mass	Container	Label
Per participant			
Sodium hydroxide (2 M)	15 mL	Plastic test tube (15 mL) with cap	NaOH
Hydrochloric acid (1 M)	15 mL	Plastic test tube (15 mL) with cap	HCl
Alkaline sodium–potassium tartrate solution	15 mL	Plastic test tube (15 mL) with cap	$KNaC_4H_4O_6$
Copper(II) sulfate (0.5 M)	15 mL	Plastic test tube (15 mL) with cap	$CuSO_4$
Sodium nitrite (0.1 M)	15 mL	Plastic test tube (15 mL) with cap	$NaNO_2$
Iron(III) chloride (0.1 M)	15 mL	Plastic test tube (15 mL) with cap	$FeCl_3$
β-Naphthol, alkaline solution	15 mL	Plastic test tube (15 mL) with cap	β-naphthol
Sodium salicylate	~ 0.1 g	Eppendorf tube	Drug #1
Sodium benzoate	~ 0.1 g	Eppendorf tube	Sodium benzoate
Acetylsalicylic acid	~ 0.1 g	Eppendorf tube	Drug #2
N-(4-Hydroxyphenyl)acetamide (paracetamol)	~ 0.1 g	Eppendorf tube	Drug #3
D-(−)-Threo-1-(p-nitrophenyl)-2-dichloroacetamido-1,3-	~ 0.1 g	Eppendorf tube	Drug #4

propanediol (chloramphenicol)			
Phthalylsulfathiazole (phthalazol)	~ 0.1 g	Eppendorf tube	Drug #5
p-Aminobenzoic acid	~ 0.1 g	Eppendorf tube	Drug #6 *p*-aminobenzoic acid
Unknown substances 1 and 2	~ 0.1 g	Eppendorf tube	**1, 2**
Mixture of unknown substances	~ 0.1 g	Eppendorf tube	**3**
Common reagents			
Zinc metal	Several granules	100 mL beaker	Zn
pH indicator paper			

Equipment	Quantity
Per participant	
12-well reaction plate	1

In the pharmacopeia, each active pharmaceutical ingredient is accompanied by a test for identity—a specific reaction that confirms its presence in a dosage form. Identity testing is crucial for quality control during drug manufacture and storage. Note that pharmaceutical formulations often include not only the active compound but also excipients—such as glucose, starch, or neutral salts— those that have no pharmacological effect. Therefore, in your analysis you must distinguish reactions characteristic of the active ingredient from those involving excipients.

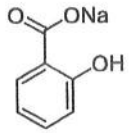
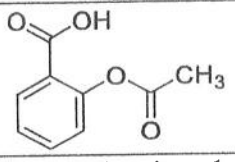
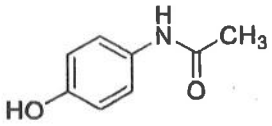
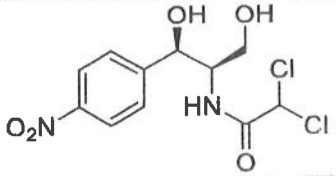
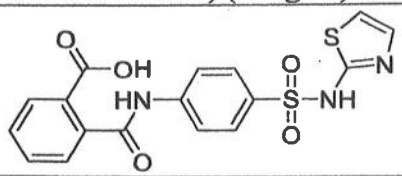
The task is divided into three subparts:

1) First, you will perform detection reactions using the known substances.
2) Next, using these reactions, you will identify two unknown individual substances.
3) Finally, you will conduct a qualitative analysis of a mixture of two unknown substances.

Reactions may be carried out in test tubes (with or without heating) or in the 12-well plate (without heating).

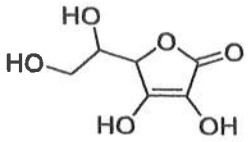
Subpart 1 You are provided with a set of reagents listed below. Use them both as reagents and to practice the detection reactions for the unknown substances:

Solutions: sodium hydroxide (2 M); hydrochloric acid (1 M); alkaline sodium–potassium tartrate; copper(II) sulfate (0.1 M); alkaline β-naphthol solution; sodium nitrite (0.1 M); iron(III) chloride (0.1 M).
Solids: metallic zinc; sodium benzoate.
Indicator paper.

Pharmaceutical standards	
Sodium salicylate (Drug #1)	Acetylsalicylic acid (aspirin; anti-inflammatory) (Drug #2)
	
N-(4-Hydroxyphenyl)acetamide (paracetamol; antipyretic) (Drug #3)	D-(−)-Threo-1-(p-nitrophenyl)-2-dichloroacetamido-1,3-propanediol (chloramphenicol; antibacterial) (Drug #4)
	
Phthalylsulfathiazole (phthalazol; intestinal antibacterial) (Drug #5)	*p*-Aminobenzoic acid (Drug #6)
	

Note. Remember that some of the provided substances contain excipients in addition to the active compound.

Subpart 2 You are given two **unknown** substances (Eppendorf tubes **1** and **2**) chosen from the following list of **ten compounds**. Identify each substance and support your choice.

1. Na_2CO_3	5. Sodium benzoate
2. Glucose	6. Paracetamol
3. Ascorbic acid 	7. Acetylsalicylic acid 8. Chloramphenicol 9. Phthalazol
4. Sodium salicylate	10. *p*-Aminobenzoic acid

Comments and recommendations for the detection reactions:

- Hydrolysis of a pharmaceutical can be performed by heating its acidified solution in a boiling water bath for 5 min.
- Phthalazol can be hydrolyzed in acidic medium upon heating to yield 2-(*p*-aminobenzene-sulfamido)thiazole.
- Amines may be diazotized by mixing with nitrite in acidic medium (solution must not be hot), then coupled in alkaline medium by adding β-naphthol; compare the color to a control without the analyte.
- Nitro groups can be reduced with zinc metal in acidic medium upon heating for 10 min.
- Copper(II) is reduced by sugars upon heating in alkaline medium (e.g., with tartrate solution).
- Phenols form colored complexes with iron(III) salts.
- Some solutions turn yellow and then darken upon heating with alkali for several minutes.

4-1. Circle the number of the structure of the substance in test tube 1. (5 points).
4-2. Circle the number of the structure of the substance in test tube 2 (5 points).

Subpart 3 You are given a preparation that is a mixture of **two** compounds from the ten "unknown substances" (Eppendorf #**3**). Identify both components.
The possible components are:
Component 1: Na_2CO_3; ascorbic acid; *p*-aminobenzoic acid; acetylsalicylic acid; paracetamol.
Component 2: glucose; sodium benzoate; sodium salicylate; phthalazol; chloramphenicol.

4-3. Mark the numbers of the two structures contained in mixture 3. (10 points).

Table for recording detection reactions results

No.	Substance	Reagent						
1	Na_2CO_3							
2	Glucose							
3	Ascorbic acid							
4	Sodium salicylate							
5	Sodium benzoate							
6	Paracetamol							
7	Acetylsalicylic acid							
8	Chloramphenicol							
9	Phthalazol							
10	*p*-Aminobenzoic acid							

(not checked or evaluated)