Preparatory problems

45th International Chemistry Olympiad

(IChO-2013)

Chemistry Department

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PREFACE

Dear friends!

We are happy to present you the Booklet of Preparatory problems. Members of the Science Committee really did their best to prepare interesting tasks. The set covers all major parts of modern chemistry. All the tasks can be solved by applying a basic knowledge of chemistry, even in case a problem refers to a topic of advanced difficulty. Still, we expect it will take some time and efforts of yours to find the correct answers. Thus, most probably we know how you will spend some of your time in the coming months. We wish you much pleasure while working with this set of problems.

FACE YOUR CHALLENGE, BE SMART!

Note to mentors

In addition to the problems, you will find in the Booklet:

- The list of topics of advanced difficulty
- The Safety rules and recommendations set by the IChO International Jury
- The hazard warning symbols, their designations and explanations, R-ratings and S-provisions

Worked solutions will be posted at the website by the end of May, 2013.

We pay great attention to safety. In the section preceding the practical preparatory problems you will find safety precautions and procedures to be followed. At the registration in Moscow we will ask every head mentor to sign a form stating that his/her students are aware of the safety rules and adequately trained to follow them. Prior to the Practical Examination all students will have to read and sign safety instructions translated into their languages of choice.

Few chemicals mentioned in the practical preparatory problems are classified to T+ (very toxic). It is not necessary to use these particular substances; you can search for appropriate substitutions. We would like to stress that students' training should be aimed at mastering specific laboratory skills rather than working with definite compounds. We assure you that during the Practical Examination at the 45th IChO **VERY TOXIC chemicals will be used under NO circumstances.**

Despite our great proof reading efforts, some mistakes and misprints are still possible. We appreciate your understanding and will be happy to get your feedback. Please address your comments to secretary@icho2013.chem.msu.ru. You may also write your comments on our website. Please explore our official website on a regular basis, since corrections/upgrades of the preparatory problems, if any, will posted there.

Acknowledgements

We would like to express our deep gratitude to Prof. A. Shevelkov, Prof. V. Nenaidenko, and Dr. Yu. Halauko as well as to the members of the International Steering Committee for their valuable comments and suggestions.

Sincerely yours,
Members of the IChO-2013 Science Committee

Contents

Physical Constants, Formulas and Equations

Avogadro's constant: $N_A = 6.0221 \times 10^{23} mol^{-1}$

Universal gas constant: $R = 8.3145\ J \cdot K^{-1} \cdot mol^{-1}$

Speed of light: $c = 2.9979 \times 10^{8} m \cdot s^{-1}$

Planck's constant: $h = 6.6261 \times 10^{-34}\ J \cdot s$

Faraday's constant: $F = 96485\ C \cdot mol^{-1}$

Standard pressure, $p^\circ = 1$ bar $= 10^5$ Pa

Zero of the Celsius scale, 273.15 K

1 nanometer (nm) $= 10^{-9}$ m

1 electronvolt (eV) $= 1.6022 \cdot 10^{-19}$ J $= 96485\ J \cdot mol^{-1}$

Energy of light quantum with wavelength λ: $E = hc / \lambda$

Energy of one mole of photons: $E = hcN_A / \lambda$

Gibbs energy: $G = H - TS$

Relation between equilibrium constant, standard electromotive force and standard Gibbs energy:

$$K = \exp\left(-\frac{\Delta G^\circ}{RT}\right) = \exp\left(\frac{nFE^\circ}{RT}\right)$$

Clapeyron equation for phase transitions: $\frac{dp}{dT} = \frac{\Delta H}{T\Delta V}$

Clausius-Clapeyron equation for phase transitions involving vapor: $\frac{d \ln p}{dT} = \frac{\Delta H}{RT^2}$

Dependence of Gibbs energy of reaction on concentrations: $\Delta G = \Delta G^\circ + RT \ln \frac{c_{prod}}{c_{reag}}$

Dependence of electrode potential on concentrations: $E = E^\circ + \frac{RT}{nF} \ln \frac{c_{ox}}{c_{red}}$

Topics of advanced difficulty

Theoretical

1. Simple phase diagrams, the Clapeyron and Clausius-Clapeyron equations, triple points.
2. Analysis of complex reactions using steady-state and quasi-equilibrium approximations, mechanisms of catalytic reactions, determination of reaction order for complex reactions.
3. Relation between equilibrium constants, electromotive force and standard Gibbs energy; dependence of Gibbs energy on the reaction mixture composition (isotherm of chemical reaction).
4. Biosynthesis of peptides and proteins: translation, genetic code, canonical amino acids, mRNA and tRNA, codone-anticodone interaction, aminoacyl tRNA synthetases.
5. Reactions of monocyclic homo- and heterocycles with less than 7 carbon atoms in the ring.
6. Redox reactions of hydroxyl, ketone and aldehyde groups.

Practical

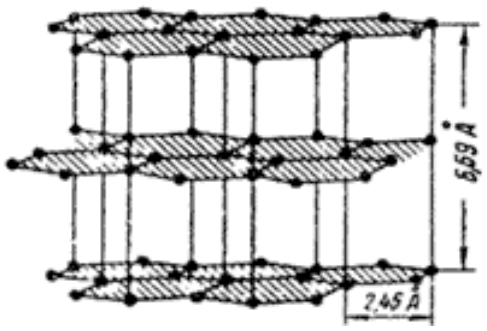
1. Conductometry
2. Viscometry

Whilst it is not explicitly stated in the Regulations, we expect the students to be acquainted with basic synthetic techniques: vacuum filtration, drying of precipitates, determination of melting point and extraction with immiscible solvents.

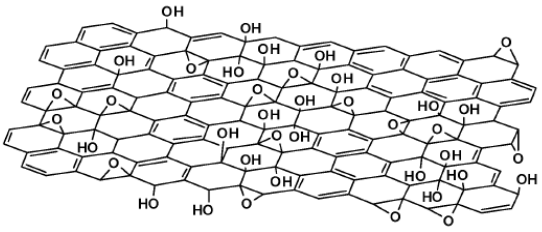
Theoretical problems

Problem 1. Graphite oxide

Graphite oxide (GO) is a compound obtained by treating graphite with strong oxidizers. In GO carbon honeycomb layers (Fig. 1a) are decorated with several types of oxygen containing functional groups. A net molecular formula of GO is CO_XH_Y, where X and Y depend on the method of oxidation. In recent years GO has attracted much attention as a promising precursor of graphene, the most famous two-dimensional carbon nanomaterial with unique electrical properties. The exfoliation of graphite oxide produces atomically thin graphene oxide sheets (Fig. 1b). The reduction of the latter produces graphene.



a)



b)

Figure 1. a) Crystal lattice of graphite. GO retains the layer structure of graphite, but the interlayer spacing is almost two times larger (~12 Å instead of 6.69 Å in the figure) and part of the carbon atoms are oxidized. b) Single sheet in the GO crystal lattice. Several oxygen containing functional groups are shown. Absolute and relative number of functional groups depends on the particular synthesis method.

1. Give two reasons why GO is more favorable precursor of graphene, compared to graphite itself? What in your opinion is the most serious disadvantage of GO as a graphene precursor?

2. The simplest model of the GO sheet (the Hoffman model) is presented in Fig. 2a. It was assumed that only one functional group, namely (–O–) is formed in the carbon plane as a result of the graphite oxidation. Calculate X in the net formula CO_X of GO, if 25% of carbon atoms in GO keep the sp^2 hybridization. What is the maximum X in the Hoffman model?

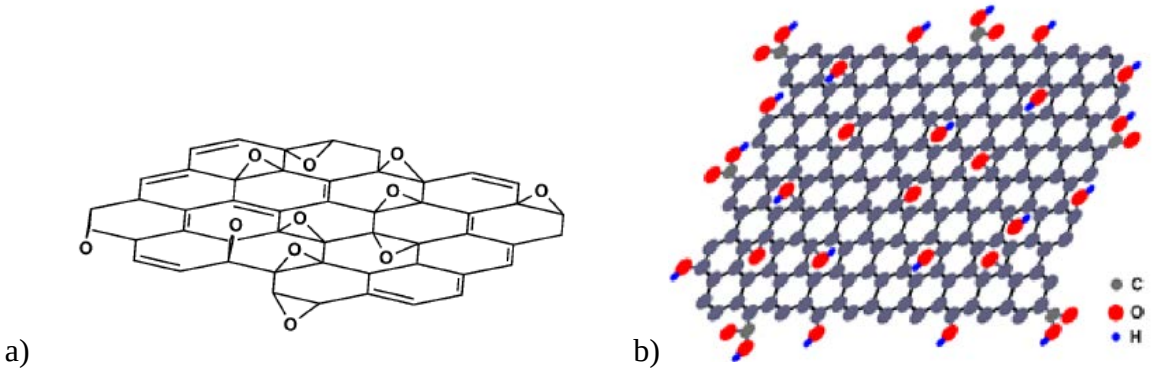


Figure 2. (a) Hoffman structural model of the GO sheet/ (b) Lerf-Klinowski model

3. The up-to date model of a single GO sheet (Lerf-Klinowski model) is shown in Fig. 2b. Name functional groups shown in the Figure.

4. Let all the sheets in a GO lattice look like it was predicted in the Lerf-Klinowski model (Fig. 2b). The net formula of the material is $CH_{0.22}O_{0.46}$. Estimate the amount of carbon atoms (in %) which were not oxidized. Give the upper and lower limits.

5. GO absorbs water in between the GO sheets. This is one of the most important properties of the material. Absorption occurs due to the formation of hydrogen bonds between molecules of water and functional groups (Fig. 3). Let GO have the net formula $CH_{0.22}O_{0.46}$. What maximum amount of water molecules can be absorbed per atom of carbon in this case? What is the net formula of the corresponding GO hydrate? Use the Lerf-Klinowski model. Consider only contacts depicted in Fig.3 (one molecule of water between two epoxy and/or between two OH groups).

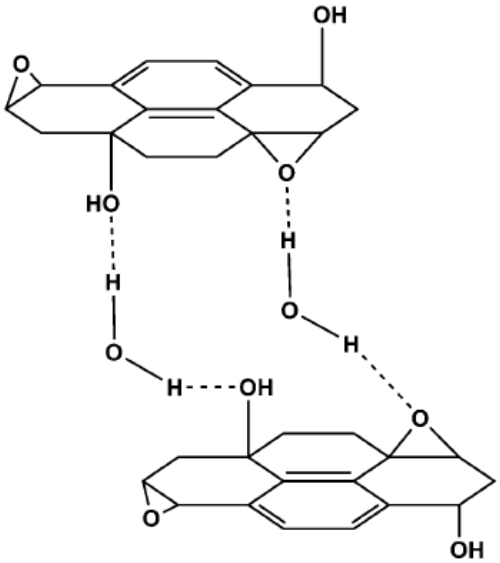


Figure 3. Proposed hydrogen bonding network formed between oxygen functionality on GO and water

Problem 2. Efficiency of photosynthesis

Photosynthesis is believed to be an efficient way of light energy conversion. Let's check this statement from various points of view. Consider the overall chemical equation of photosynthesis performed by green plants in the form:

$$H_2O + CO_2 \rightarrow CH_2O + O_2$$

where CH_2O denotes the formed carbohydrates. Though glucose is not the main organic product of photosynthesis, it is quite common to consider CH_2O as 1/6(glucose). Using the information presented below, answer the following questions.

1. Calculate the standard enthalpy and standard Gibbs energy of the above reaction at 298 K. Assuming that the reaction is driven by light energy only, determine the minimum number of photons necessary to produce one molecule of oxygen.

2. Standard Gibbs energy corresponds to standard partial pressures of all gases (1 bar). In atmosphere, the average partial pressure of oxygen is 0.21 bar and that of carbon dioxide – $3 \cdot 10^{-4}$ bar. Calculate the Gibbs energy of the above reaction under these conditions (temperature 298 K).

3. Actually, liberation of one oxygen molecule by green plants requires not less than 10 photons. What percent of the absorbed solar energy is stored in the form of Gibbs energy? This value can be considered as the efficiency of the solar energy conversion.

4. How many photons will be absorbed and how much biomass (in kg) and oxygen (in m^3 at 25 ^oC and 1 atm) will be formed:

a) in Moscow during 10 days of IChO;

b) in the MSU campus during the practical examination (5 hours)?

5. What percent of the solar energy absorbed by the total area will be converted to chemical energy:

a) in Moscow;

b) in MSU?

This is another measure of photosynthesis efficiency.

Necessary information:

Average (over 24 h) solar energy absorbed by Moscow region in summer time – 150 $W \cdot m^{-2}$;

Moscow area – 1070 km^2, percentage of green plants area – 18%;
MSU campus area – 1.7 km^2, percentage of green plants area – 54%;
green plants utilize ~10% of the available solar energy (average wavelength is 680 nm)

Substance	$H_2O_{(l)}$	$CO_{2(g)}$	$O_{2(g)}$	$C_6H_{12}O_{6(s)}$
Standard enthalpy of combustion, $\Delta_c H^\circ_{298}$, kJ·mol^{-1}	–	–	–	–2805
Standard entropy, S°_{298}, J·K^{-1}·mol^{-1}	70.0	213.8	205.2	209.2

Problem 3. Ammine complexes of transition metals

1. The synthesis of chromium(3+) ammine complexes usually starts from a freshly prepared *in situ* solution of a chromium(2+) salt. How can one prepare such a solution using metallic chrome? Specify the conditions.

2. To the solution of a chromium(2+) salt, the solution of ammonia and a solid ammonium chloride are added. Then a stream of air is passed through the solution. The red precipitate is formed that contains 28.75 wt.% of N. Determine the composition of the precipitate and give the reaction equation.

3. What oxidizer can be used instead of oxygen to obtain the same product? Justify the choice.

4. What product will be formed if the experiment described above is performed under inert atmosphere without oxygen? Give the equation.

5. Explain why the ammine complexes of chromium(3+) cannot be prepared by the action of water ammonia on a solution of chromium(3+) salt.

6. Arrange the hexammine complexes of iron(2+), chromium(3+) and ruthenium(2+) in a row of increasing stability towards the acidic water solutions. Explain your choice.

7. In the case of $[Ru(NH_3)_6]^{2+}$ the hydrolysis rate increases upon the addition of an acid. Propose a mechanism and derive the rate law.

Problem 4. Preparation of inorganic compound

The substance X has been prepared by the following procedures. Copper(II) sulfate pentahydrate (ca 10 g) was dissolved in a mixture of distilled water (80 cm^3) and concentrated sulfuric acid (4 cm^3). The solution was boiled with analytical-grade metallic tin (10 g) until the solution became colorless and the deposited copper was covered with a grey coating of tin. The resultant solution was filtered and treated with an ammonia-water solution until the complete precipitation of a product. It was filtered off and washed with water until no odor of ammonia was detectable. The precipitate obtained was added to the nitric acid solution gradually in small portions, with stirring, until the solution was saturated. The suspension was boiled for 2 min, filtered into a warm, insulated flask and allowed to cool slowly. The 1.05 g of crystalline product X was obtained. Under heating X rapidly decomposes with the mass loss of 17.49%. The residue formed is a binary compound identical with the common mineral of tin. The volatile decomposition products passed over 1.00 g of anhydrous copper(II) sulfate increase its mass by 6.9%.

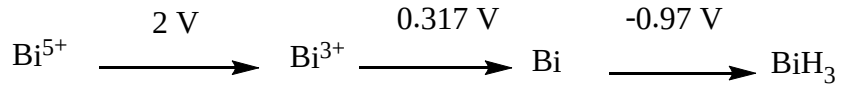
1. Determine the composition of X.

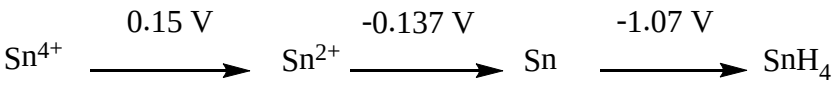
2. What important instruction has been omitted in the description of the procedure?

3. Predict the structure of the cation in X taking into account that all the metal atoms in it are equivalent.

4. What particles are formed by addition of an acid or an alkali to the solution of X?

5. What happens when 1 M solution of bismuth trichloride in 1 M HCl is added to the 1 M solution of tin chloride? Calculate the equilibrium constant of the reaction. Extract the necessary data from the Latimer diagrams below.





Problem 5. Inorganic chains and rings

1. The interaction of thionyl chloride and sodium azide at –30°C gives colorless crystals X, containing 36.4 wt.% of Cl. The crystals consist of cyclic trimers. Find the composition of X and give the reaction equation.

2. Draw two stereoisomers of X.

3. A colorless liquid Y was prepared by a reaction between X and antimony(III) fluoride. Addition of 1.00 g of Y to the excess of barium acetate aqueous solution gave the precipitate with the mass of 3.96 g. Determine the chemical formula of Y, draw its structure and write the reaction equation.

4. Y enters the substitution reactions with typical nucleophiles such as methylamine. What product will be formed in the reaction between Y and the excess of methylamine? Draw its structure.

5. Give two examples of molecules or ions which are isoelectronic to Y, draw their structures.

6. One of the substances isoelectronic to Y transforms in the presence of water traces into polymer Z. 1.00 g of Z was dissolved in water and the resulting solution was added to the excess of barium acetate solution. The precipitate with the mass of 2.91 g was formed. Determine the formula of Z and draw its structure.

Problem 6. Transition metal compounds

Procedures for the synthesis of several compounds of transition metal **X** are given below.

"A solution of 2 g of very fine powder **A** in 50 mL of 28% sodium hydroxide is triturated in a small Erlenmeyer flask with 3.5 g of finely ground $Na_2SO_3 \cdot 7H_2O$; the flask stands in an ice bath. The trituration requires about 10 minutes, that is, until a light-blue crystalline slurry is obtained. The mixture is then transported under vacuum onto an ice-cooled glass filter, and the product washed thoroughly with 28% sodium hydroxide at 0°C. The wet preparation is rapidly spread in a thin layer on fresh clay and stored at 0°C in an evacuated desiccator (no drying

agent)… The preparative procedure should be designed so as to avoid contamination by silicates or aluminates … Product **B**, in the form of well-crystallized sky-blue rods, remains stable at 0°C if kept free of H_2O and CO_2… A solution of **B** in 50% potassium hydroxide turns grassy green upon heating or dilution; simultaneously, **C** is precipitated.

In a pure form, salt **D**, which is a main constituent of **B**, is prepared according to the following procedure: «NaOH is entirely dehydrated by heating in silver pot at 400ºC and mixed with **C** in a such way that Na : **X** molar ratio is 3 : 1. Mixture is heated to 800ºC in a silver pot and kept under oxygen flow for 5 h. The formed product **D** is rapidly cooled to room temperature». Salt **D** is a dark-green compound inert to CO_2.

A solution of 30 g of KOH in 50 mL of water is prepared; 10 g of **A** is added and the mixture is boiled in an open 250-mL Erlenmeyer flask until a pure green solution is obtained. The water lost by evaporation is then replaced and the flask set in ice. The precipitated black-green crystals, which show a purplish luster, are collected on a Pyrex glass filter, washed (high suction) with some 1 M potassium hydroxide, and dried over P_2O_5. The formed compound **E** can be recrystallized by dissolving in dil. KOH and evaporated in vacuum».

1. Determine the element **X** and molecular formulae of **A**-**E** using the following data: a) sodium weight content in **B** is 18.1%; b) the weight content of the element **X** in **A**, **B**, **C**, **D**, and **E** is 34.8, 13.3, 63.2, 29.3, and 27.9% respectively.

2. Write all the reaction equations.

Problem 7. Simple equilibrium

The gaseous substances A_2 and B_2 were mixed in a molar ratio 2:1 in a closed vessel at a temperature T_1. When the equilibrium $A_2(g) + B_2(g) = 2AB(g)$ was established the number of heteronuclear molecules in a gas phase became equal to the total number of homonuclear molecules.

1. Determine the equilibrium constant K_1 for the above reaction.

2. Find the ratio of heteronuclear to homonuclear molecules at equilibrium if the substances are mixed in a ratio 1:1 at the temperature T_1?

The equilibrium mixture obtained from the initial mixture $A_2 : B_2 = 2 : 1$ was heated so that equilibrium constant became $K_2 = K_1 / 2$.

3. How much substance B_2 (in percent to the initial amount) should be added to the vessel in order to keep the same equilibrium amounts of A_2 and AB as at the temperature T_1?

Consider the reaction yield $\eta = n_{eq}(AB) / n_{max}(AB)$ as a function of the initial molar ratio $A_2 : B_2 = x : 1$ at any fixed temperature (n_{max} is the maximum amount calculated from the reaction equation). Answer the following questions qualitatively, without exact equilibrium calculations.

4. At what x the yield is extremal (minimal or maximal)?

5. What is the yield at: a) $x \to \infty$; b) $x \to 0$?

6. Draw the graph of $\eta(x)$.

Now, consider the variable ratio $A_2 : B_2 = x : 1$ at a fixed total pressure.

7. At what x the equilibrium amount of AB is maximal?

Problem 8. Copper sulfate and its hydrates

A British artist Roger Hiorns entirely filled a flat with a supersaturated copper sulfate solution. After removal of the solution, blue crystals remained on the walls, floor, and ceiling.

1. Write down the formula of these crystals.

2. Humidity inside this flat has a constant low level. Using the Clausius-Clapeyron equation, calculate the temperature at which the humidity will be 35% (of the saturated vapor pressure of water at the same temperature).

Copper sulfate is often used in laboratories as a drying agent, for example, to obtain absolute ethanol.

3. By rectification of aqueous ethanol one can increase its concentration to not more than 95.5 wt.%. This is due to the fact that:

a) pressures of water and ethanol vapor are the same

b) mole fractions of ethanol in the gas and liquid phases are equal

c) water forms a stable complex with ethanol

d) ethanol absorbs water vapor from the air

Choose the correct answer.

For further dehydration of ethanol, anhydrous copper sulfate is added. After a while the liquid is decanted and treated with a new portion of anhydrous copper sulfate. These operations are repeated 2-3 times until copper sulfate will stop turning blue. Then ethanol is filtered and distilled.

4. What is the minimum residual water content (in mass percent) that can be achieved by using this method at room temperature?

Two chemists argued at what temperature – high or low – should the process of drying be performed in order to achieve lower residual water content.

5. Calculate the minimum residual water contents if ethanol was dried at 0 °C and 40 °C.

Necessary information. Vapor pressure of water over its dilute solution in ethanol is given by $p = p_{sat}\gamma x$, where p_{sat} is the saturated vapor pressure of water, x is the mole fraction of water in solution, γ is the activity coefficient of water, which only slightly depends on temperature and can be assumed to be 2.45.

	$\Delta_f H^\circ_{298}$ / (kJ·mol^{-1})	p_{sat} / Pa at 298K
$CuSO_4 \cdot 5H_2O$	–2277.4	1047
$CuSO_4 \cdot 3H_2O$	–1688.7	576
$CuSO_4 \cdot H_2O$	–1084.4	107
$CuSO_4$	–770.4	
H_2O (l)	–285.83	3200
H_2O (g)	–241.83	

Problem 9. TOF and TON

TOF, turnover frequency, and *TON*, turnover number, are two important characteristics of a catalyst. According to the definitions given by the International Union of Pure and Applied Chemistry (IUPAC), *TOF* is the maximum number of molecules of a reagent that a catalyst can convert to a product per catalytic site per unit of time. *TON* is the number of moles (or molecules) of a reagent that a mole of catalyst (or a catalytic site) can convert before becoming inactivated. *TON* characterizes the stability (life time) of a catalyst, while *TOF* is a measure of its best efficiency. Very important is the word "maximum" in the definition of *TOF*!

In Russian, *TOF* and *TON* sound like names of two clowns

1. *TON* is a dimensionless value. What is the dimension of *TOF*? Derive a relation between *TON* and *TOF*.

2. Let a catalytic reaction A + Cat → B proceed in a closed system. A and B are gases, Cat is a solid catalyst.

a) The dependence of the amount of B produced at 1 cm^2 of a catalytic surface upon time is given in Fig. 1a. There are 10^{15} catalytic sites in 1 cm^2 of the surface. Estimate *TOF*.

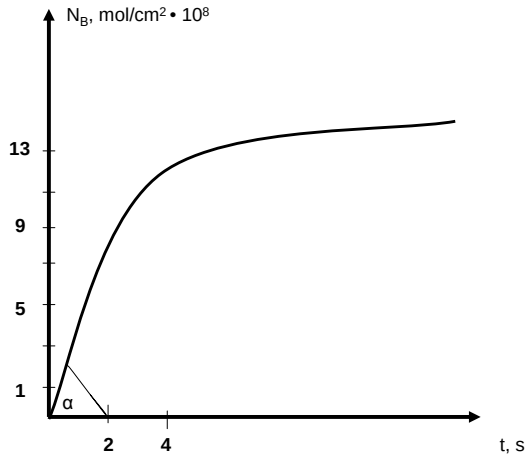


Figure 1a. The amount of the product N_B as a function of time

b) The dependences of the amount of B formed in 1 cm^2 of the catalytic surface upon time are given in Fig. 1b. Different curves correspond to different initial pressures of the reagent A. These pressures (in arbitrary units) are shown by red numbers. There are 10^{15} catalytic sites in 1 cm^2 of the surface. Calculate *TOF* for the catalyst. This catalyst worked during 40 minutes and then became inactivated. Estimate *TON*.

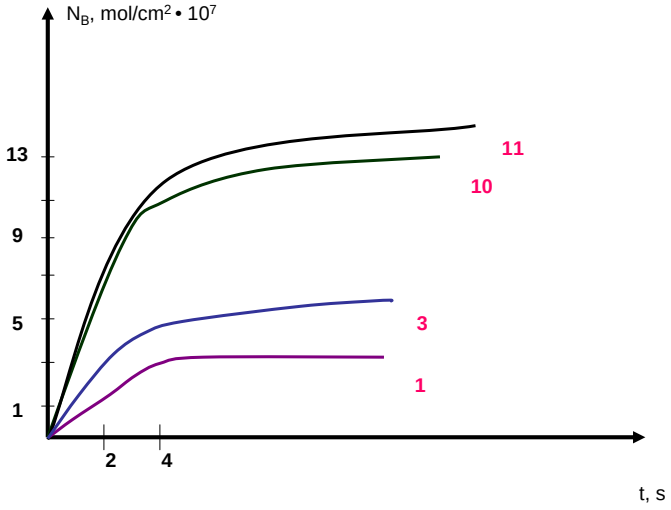


Figure 1b. The amount of the product N_B as a function of time

3. a) *TOF* is often used to describe the operation of deposited catalysts. To make a deposited catalyst one has to deposit atoms of metal on the inert surface. These atoms form catalytic sites. The dependence of the rate of the catalytic reaction upon the amount of metal atoms deposited on 1 cm^2 of the surface (less than one monolayer) is shown in Fig. 2a. Calculate TOF.

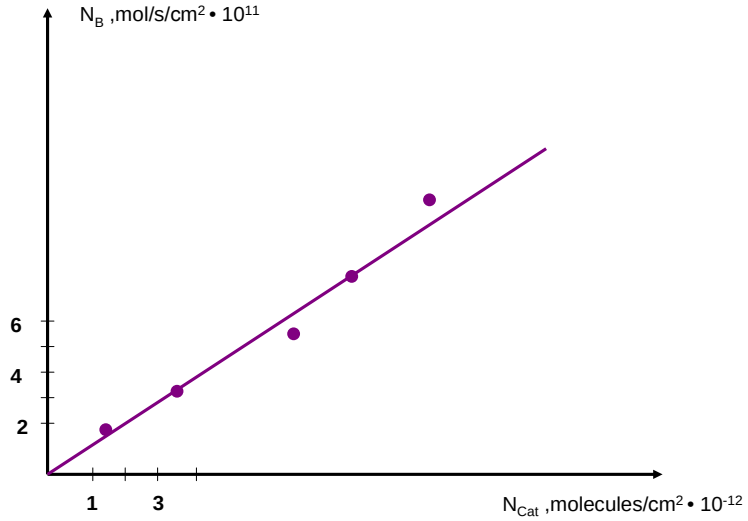


Figure 2a. The dependence of N_b on N_{Cat}

b) Russian scientist professor Nikolay I. Kobozev has shown that the dependence of N_B on N_{Cat} can be much more complicated. The corresponding curve in Fig. 2b has maximum! According to the Kobozev's theory (a simplified version) a structure consisted of n deposited atoms rather than a single atom form a catalytic site. Maximum rate of catalytic reaction was observed when

$$\frac{(\text{number of deposited atoms per surface unit})}{(\text{number of catalytic sites per surface unit})} = n$$

From the data shown in Fig. 2b calculate n, the number of atoms forming a catalytic site. *TOF* for the point of maximum rate in Fig. 2b is given in SI units.

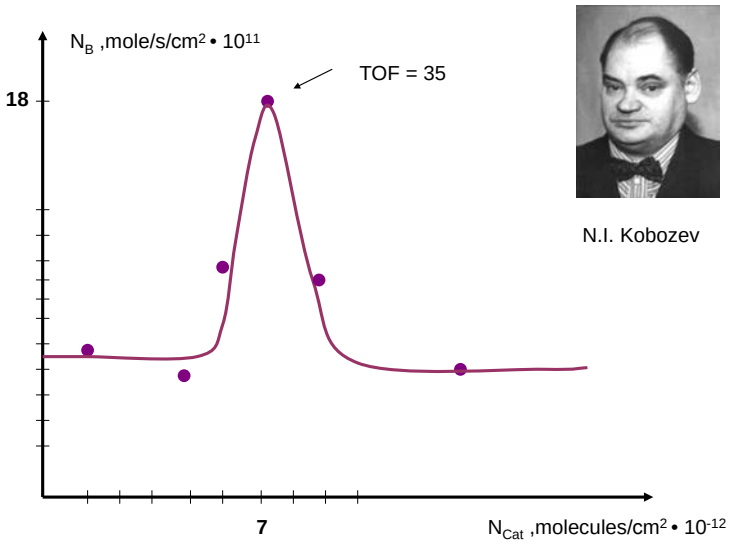


Figure 2b. The dependence of N_b on N_{Cat}

4. Atoms of Au deposited on the Mo-TiO_X support exhibit exceptional catalytic activity for the CO oxidation

$$CO + 0.5O_2 \xrightarrow{Au} CO_2$$

(M. S. Chen and D. W. Goodman, Science, v.306, p.254, 2004).

The maximum rate of reaction r_1 {mol/cm^2/s} was observed for the bilayer atomic structure presented in Fig. 3a. Red and yellow spheres are atoms of Au. For the monolayer structure (Fig. 3b), the reaction was four times slower, r_2 = ¼ r_1. Calculate the ratio of *TOF* for the atoms of Au in the upper layer in Fig. 3a (all red spherical particles), to *TOF* for the monolayer in Fig. 3b (all yellow spherical particles). In the former case, every single Au atom is a catalytic site. The rate of the catalytic reaction on each yellow site in Fig.3a and Fig.3b is the same if the site is accessible to reactants and is equal to zero if the access is blocked.

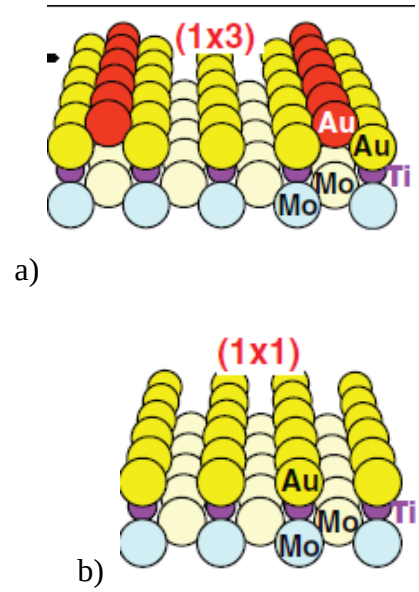


Figure 3. Structure of the gold catalyst deposited on the Mo-TiO_2 support.
a) Bilayer structure; b) monolayer structure

Problem 10. Kinetic puzzles

Propose the mechanisms for the reactions given below. Prove that your mechanisms are consistent with the experimentally observed rate laws. Use proper approximations if necessary.

1. Oxidation of bromide ion by permanganate in acidic solution

$$2MnO_4^- + 10Br^- + 16H^+ = 2Mn^{2+} + 5Br_2 + 8H_2O$$

a) at low concentrations of Br^- and H^+

$$r = kc(MnO_4^-)c^2(Br^-)c^3(H^+)$$

b) at high concentrations of Br^- and H^+

$$r = kc(MnO_4^-)c(Br^-)c(H^+)$$

where c are the total concentrations of reactants. In both cases $c(MnO_4^-) << c(Br^-)$, $c(H^+)$.

2. Oxidation of benzamide by peroxydisulfate in the presence of Ag^+ ions in water-acetic acid solution

$$2C_6H_5CONH_2 + 2H_2O + 3S_2O_8^{2-} = 2C_6H_5COOH + 6SO_4^{2-} + N_2 + 6H^+$$

$$r = k[Ag^+][S_2O_8^{2-}]$$

3. Oxidation of formate ion by peroxydisulfate in water solution

$$HCOO^- + S_2O_8^{2-} = CO_2 + 2SO_4^{2-} + H^+$$

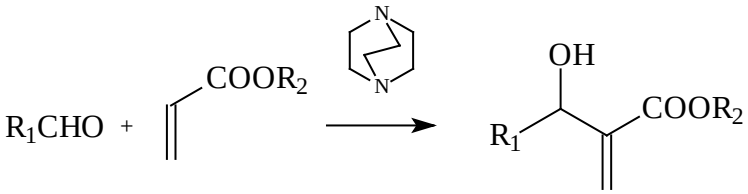
$$r = k[HCOO^-]^{1/2}[S_2O_8^{2-}]$$

4. Oxidation of azide ion by iodine in carbon disulfide solution

$$I_2 + 2N_3^- = 3N_2 + 2I^-$$

$$r = k[N_3^-]$$

5. Condensation of aldehydes with acryl esters in the presence of the base – 1,4-diazabicyclo[2.2.2]octane (DABCO) in tetrahydrofurane solution



$$r = k[\text{aldehyde}]^2[\text{ester}][\text{DABCO}]$$

6. Decomposition of peroxyacids in water solution

$$2RCO_3H = 2RCO_2H + O_2$$

$$r = c^2(RCO_3H)\frac{k_1[H^+]}{(k_2 + [H^+])^2},$$

where $c(RCO_3H)$ is the total concentration of acid. Consider the following: when the mixture of normal RCO–O–O–H and isotopically labeled RCO–^{18}O–^{18}O–H peroxyacid is used as a reactant, the main species of evolving oxygen are $^{16}O_2$ and $^{18}O_2$.

Problem 11. Black box

Substance P is synthesized from substances X and Y in a constant-flow reactor which has two feeds for reagent solutions and one outlet for a resulting solution (all solutions are liquid). The operator of the reactor can set flows of the reagents at his will. Due to intensive stirring the concentration of any substance is the same in any part of the reactor. The measured parameters of the working reactor are given in the table below.

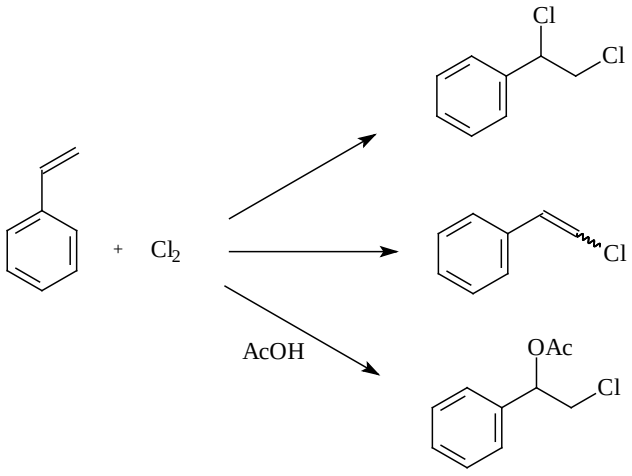
Exp. no.	Input flow of reactant solutions, m^3/s		Concentrations of reactants in input flows, mol/m^3		Concentrations of substances in output flow, mol/m^3		
	X	Y	X	Y	X	Y	P
1	0.0100	0.0100	1600	2100	299	48.2	501
2	0.0200	0.0100	1600	2100	732	30.9	335
3	0.0100	0.0200	1600	2100	8.87	351	524
4	0.0200	0.0200	1600	2100	308	66.6	492

Using the data above, obtain as much information as possible about this system, e.g. the volume of the reactor, the reaction rate constant, the reaction orders, etc. If you find the reaction orders, propose a mechanism which is consistent with the discovered rate law.

Hint: because the reaction proceeds in a liquid phase, the output volumetric flow is equal to the sum of input volumetric flows.

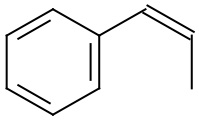
Problem 12. Chlorination of styrenes

Addition of chlorine to styrenes is often accompanied by the formation of 2-chlorostyrene. In some solvents, the formation of solvent-incorporated products is also observed. For example, chlorination of styrene in acetic acid yields a 1-acetoxy-2-chloro derivative. The overall process can be illustrated by the following scheme:



Formation of each product obeys the same rate law: the reaction order is 1 with respect to both styrene and chlorine.

The product distribution during the chlorination of *cis*-1-phenylpropene



at 25^{o}C is given in the Table.

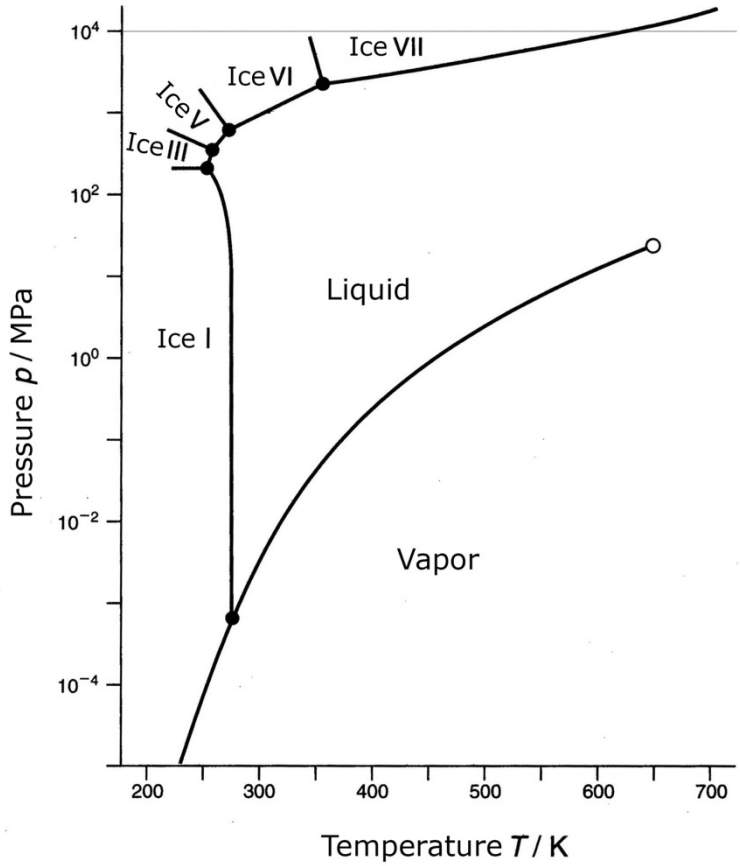
product	1,2-dichloro	1-acetoxy-2-chloro	2-chlorostyrene
mol %	61	30	9

1. The rate constant of the overall reaction is $1.45 \cdot 10^4$ $M^{-1}s^{-1}$ at 25^{o}C. What are the rate constants for the formation of 1,2-dichloro and 1-acetoxy-2-chloro adducts and 2-chlorostyrene?

2. Products of this reaction can be separated by chromatography. If the achiral sorbent is used, the determined number of products in *cis*-1-phenylpropene + chlorine reaction is 6. Why? What is the determined number of products if the sorbent is chiral?

Problem 13. The dense and hot ice

The pressure-temperature phase diagrams of pure substances describe the conditions at which various equilibrium phases exist. The phase diagram of water is shown below (pressure is given in the logarithmic scale).



The phase diagram of water in the semi-log scale

Using this diagram and the appropriate thermodynamic equations describing phase transitions, answer the following questions.

1. How do the boiling point of water and the melting points of ordinary ice (ice I) and ice V vary with pressure? Explain this qualitatively applying the Le Chatelier principle.

2. What would happen with water vapor if the pressure is gradually increased from 10 Pa to 10 GPa at a temperature: a) 250 K, b) 400 K, c) 700 K ?

3. The lowest possible temperature at which equilibrium liquid water still exists is achieved in the triple point between water, ice I, and ice III. The pressure in this point is 210 MPa, estimate the temperature.

4. Several forms of ice can exist in equilibrium with liquid water. Assuming that the heat of fusion is approximately the same for all forms, determine, which of the ices has the largest density. What is the melting point of this ice at a pressure of 10 GPa?

5. The densest ice has the cubic crystal structure with two water molecules per one unit cell. The edge of the unit cell is 0.335 nm. Calculate the density of ice.

6. Estimate the enthalpy of fusion of the densest ice.

Necessary data:

densities of ordinary ice and water: 0.917 and 1.000 g/cm^3, respectively;

enthalpy of fusion of ordinary ice: +6010 J/mol;

triple point «water – ice VI – ice VII»: pressure 2200 MPa, temperature 355 K.

Hint. Assume that the densities of condensed phases and the enthalpies of phase transitions do not vary with pressure and temperature.

Problem 14. Redox reactions in photosynthesis

Redox reactions are at the heart of photosynthesis. Some of them are spontaneous, others are driven by light or conjugated chemical reactions. The former are named exergonic ($\Delta G < 0$), the latter – endergonic ($\Delta G > 0$).

Every redox reaction consists of two conjugated processes (half-reactions) – oxidation and reduction. In photosynthesis, half-reactions are often separated not only in space, but also in time. In living organisms, this is performed by dividing redox reactions into many steps involving bioorganic substances – enzymes, cofactors, etc.

Every half-reaction is characterized by a standard redox potential $E°$ which refers to 1 M concentration of all substances in solution and 1 bar pressure of all gaseous substances. The values of $E°$ for several reactions involved in photosynthesis are listed in the table. Biochemists usually correct the standard potential to pH 7.0 and designate it as $E°'$.

Photosynthesis in green plants and algae can be described by an overall equation (see Problem 2):

$$H_2O + CO_2 \rightarrow CH_2O + O_2$$

In this process water is oxidized to O_2, and carbon dioxide is reduced to carbohydrates. The former reaction occurs under the action of light and consists of the so called light stages, the latter is driven by exergonic chemical reactions and involves the dark stages only.

Half-reaction	Standard redox potential, $E°$ (V)
$O_2 + 4H^+ + 4e \rightarrow 2H_2O$	1.23
$S + 2H^+ + 2e \rightarrow H_2S$	0.14
$Plastoquinone + 2H^+ + 2e \rightarrow Plastoquinone{\cdot}H_2$	0.52
$Cytochrome\ f(Fe^{3+}) + e \rightarrow Cytochrome\ f(Fe^{2+})$	0.365
$NADP^+ + H^+ + 2e \rightarrow NADP{\cdot}H$	–0.11
$P680^+ + e \rightarrow P680$	1.10
$Chlorophyll^+ + e \rightarrow Chlorophyll$	0.78

1. Calculate the standard biochemical redox potential for all half-reactions presented in the table above.

2. Using the answers obtained in Problem 2, determine $E°$ and $E°'$ for the half-reaction of CO_2 reduction to CH_2O.

Some bacteria convert CO_2 into organic matter, but do not produce molecular oxygen. In these organisms, other substances are oxidized instead of water, e.g. H_2S or H_2.

3. Write the overall reaction equation of photosynthesis in green sulfur bacteria, which oxidize hydrogen sulfide to elementary sulfur. Separate this equation into the oxidation and reduction steps. Calculate the standard Gibbs energy of the overall reaction at 298 K. Assuming that the reaction is driven by light energy only, determine the minimum number of photons (840 nm) necessary to oxidize one molecule of hydrogen sulfide.

Light reactions in green plants lead to the oxidation of water, reduction of $NADP^+$ to $NADP{\cdot}H$, and formation of adenosine triphosphate (ATP) from adenosine diphosphate (ADP) and HPO_4^{2-} (designated as P_i). The latter process is described by the equation:

$$ADP + P_i + H^+ \rightarrow ATP + H_2O$$

4. Write the overall reaction of light stages of photosynthesis in green plants.

During light stages, light energy is converted into chemical energy stored in ATP and NADH·H and wasted further in dark reactions, which are highly endoergic.

5. Calculate the Gibbs energy of the overall reaction describing light stages of photosynthesis given that the standard biochemical Gibbs energy for ATP formation is +30.5 kJ/mol.

Redox properties of molecules can change significantly after electronic excitation. The excited state can be both a stronger oxidant and a stronger reductant than the ground state.

6. Explain this effect qualitatively, considering excitation process as an electronic transition between HOMO and LUMO.

In all known photosynthetic organisms the excited states are strong reductants.

7. Derive the equation relating the redox potential of the excited state, redox potential of the ground state, and the excitation energy $E_{ex} = h\nu$. Using this equation, calculate the standard redox potential for the processes: $P680^+ + e \rightarrow P680^*$ (λ_{ex} = 680 nm) and $Chlorophyll^+ + e \rightarrow Chlorophyll^*$ (λ_{ex} = 680 nm), where asterisk denotes excited state.

Problem 15. Complexation reactions in the determination of inorganic ions

Reactions of complex formation are frequently used in titrimetric methods of determination of various inorganic ions. For example, fluoride forms a stable complex with aluminum(III):

$$6F^- + Al^{3+} = AlF_6^{3-}$$

In water the complex gives a neutral solution. This process can be used for the direct titration of fluoride and indirect determinations of other species.

In the first experiment, a sample solution containing fluoride was neutralized with methyl red, solid NaCl was added to saturation, and the solution was heated to 70–80°C. The titration was performed with 0.15 M $AlCl_3$ until yellow color of the indicator turned pink.

1. What process occurred at the endpoint?

2. Why heating increased the endpoint sharpness?

3. What is the purpose of adding sodium chloride?

In the second experiment, the content of calcium was determined in the following way. An excess of NaCl together with 0.500 g NaF were added to the sample, and the resulting solution was titrated with a standard 0.1000 M solution of $AlCl_3$ in the presence of methyl red. The endpoint was attained with 10.25 mL of the titrant.

4. What operation (absolutely necessary to make the determination correct!) is missing from the description of the procedure? Compare with the first experiment described above.

5. Write down the reactions taking place in this procedure.

6. Calculate the amount of calcium in the sample.

Similar principles are used in determination of silicic acid. To the neutralized colloidal solution of the sample, 0.5 g of KF was added, which was followed by introduction of HCl (10.00 mL of 0.0994 M solution) up to a definite excess. The resulting mixture was then titrated with a standard solution of alkali in the presence of phenyl red (5.50 mL of 0.1000 M NaOH was spent).

7. What chemical reaction(s) is the determination based on? Write silicic acid as $Si(OH)_4$.

8. What indicator should be used when neutralizing the sample of silicic acid before the titration? The pK_a values of indicators: methyl red, 5.1; phenol red, 8.0; thymolphthalein, 9.9.

9. Calculate the amount of silicic acid in the sample solution.

Problem 16. Malaprade reaction

Oxidation of 1-(3,4,5-trimethylphenyl)butane-2,3-diol with an excess of sodium periodate yields 3,4,5-trimethyl phenylacetaldehyde and acetaldehyde. Other α-dions, α-diols, and α-hydroxycarbonyls undergo similar type of oxidation (Malaprade reaction). However, carboxylic, ester and isolated aldehyde groups are not oxidized under these conditions.

1. Provide the structures of organic products of the reaction of periodate with glycerol and butane-1,2-diol (mixture **A**).

2. A weighed amount of mixture **A** ($m_{\mathbf{A}}$ = 1.64 g) was introduced into the reaction with an excess of periodate, and the formed aldehyde groups were titrated with potassium permanganate in an acidic medium, which required n_{Mn} = 0.14 mol equivalents of $KMnO_4$ (1/5 $KMnO_4$). Write down the reactions of permanganate in an acidic medium with the products of mixture **A** oxidation with periodate. Determine the molar composition of mixture **A**.

3. A weighed amount of an individual compound **B** containing an amino group ($m_{\mathbf{B}}$ = 105.0 mg) was dissolved in water and acidified. Then an excess of $NaIO_4$ was added. When the reaction was completed, $1.0 \cdot 10^{-3}$ mol of carboxylic groups (as part of carboxylic acids) and $1.0 \cdot 10^{-3}$ mol of ammonium ions were found in the mixture, while $8.0 \cdot 10^{-3}$ mol equivalents of MnO_4^- were spent for the permanganatometric titration of the products. Determine possible structures of **B**, if it is neither ether nor an ester. Propose a scheme for **B** oxidation with periodate using one of the suggested structures as an example.

Problem 17. Analysis of Chrome Green

Chrome Green pigment is obtained by mixing lead(II) chromate and iron(II) hexacyano-ferrate(III). A titrimetric method of Chrome Green analysis involves the following steps: an accurate weight of the pigment sample is treated with sodium carbonate solution while heating and then filtered.

1. Write down the reactions occurring on treatment of Chrome Green with carbonate. What is left on the filter?

To determine chromate, the iodometric method is used. An excess of KI is added to the acidified solution, and the released iodine is titrated with the standardized $Na_2S_2O_3$ solution in the presence of starch.

2. Write down the reactions occurring when chromate is determined by this method. Why is it not recommended to titrate dichromate directly with thiosulfate?

$Na_2S_2O_3$ solution should be standardized before using it as the titrant. The standardization is carried out against a standard $K_2Cr_2O_7$ solution in the same way as described above for the determination of chromate. If the acidity of the solution significantly exceeds 0.4 M, the reaction between dichromate and iodide induces the oxidation of iodide with atmospheric oxygen.

3. Propose a scheme for such an induced process. How would it affect the results of thiosulfate determination?

One aliquot of the filtered sample of Chrome Green solution (10.00 mL out of the total volume of 50.0 mL) was used for the iodometric determination of chromate following the procedure described above (5.01 mL of 0.0485 M $Na_2S_2O_3$ was spent).

4. Calculate the amount of lead chromate in the sample (mg $PbCrO_4$).

A reaction of chromium(VI) with $[Fe(CN)_6]^{4-}$ might occur upon adding the acid.

5. Estimate whether any analytical errors might be caused by this side reaction.

Another aliquot of the filtered solution (10.00 mL out of the total volume of 50.0 mL) was mixed with 10.00 mL of 0.0300 M solution of $K_4Fe(CN)_6$, acidified with H_2SO_4 to obtain $[H^+] \cong 1$ M and titrated by 0.00500 M $KMnO_4$ (2.85 mL was spent).

6. What reaction did occur upon acidification of the sample? Write down the reaction of titration with permanganate.

7. Calculate the amount of Turnbull's Blue in the sample (mg $Fe_3[Fe(CN)_6]_2$).

Problem 18. Chemistry of phenol

Phenol is a valuable industrial commodity for the synthesis of various materials and compounds with useful properties. Therefore, its annual production totals several million tons. The classical industrial method of phenol production is a two-stage process developed by the Soviet chemist R. Udris in 1942. First, the mixture of benzene **A** and propene **B** is compressed under heating in the presence of an acid as a catalyst. Interaction of equal amounts of **A** and **B** leads to compound **C** which is then oxidized with air followed by acidification, which finally results in two products: phenol and compound **D** also widely used in industry.

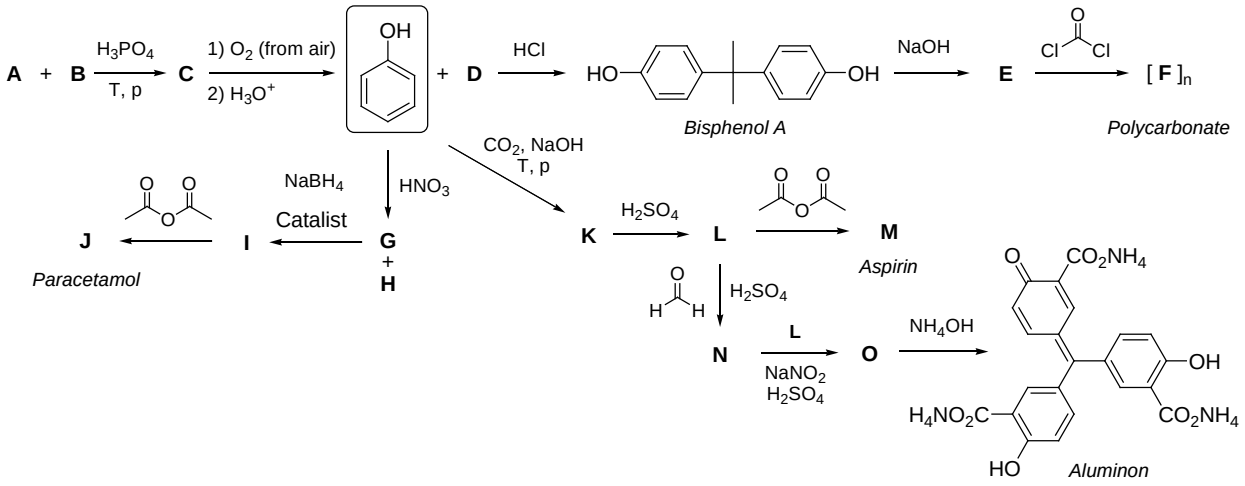
High potential of phenol in the synthesis of polymers, drugs, and dyes can be illustrated by the hereunder examples.

The reaction of phenol with **D** in the presence of an acid gives *bisphenol A*, which was for the first time synthesized by the Russian chemist A. Dianin in 1891. The treatment of *bisphenol A* with NaOH leads to **E**, which reacts with phosgene affording *polycarbonate* with a monomeric unit **F**.

The treatment of phenol with diluted nitric acid results in isomeric compounds **G** and **H**, which can be separated by steam distillation. The molecule of **G** has two planes of symmetry (that of the molecule and an orthogonal one), while the plane of the molecule is the only element of symmetry for **H**. Starting with **G**, one can obtain *paracetamol* **J** via a two-stage process.

Aspirin **M** can be obtained from phenol in three steps. First, phenol is treated with NaOH and CO_2 under heating and high pressure. This reaction gives compound **K**, which has only one element of symmetry (plane of the molecule). Two equivalents of an acid are required for acidification of **K** to form compound **L**. Further acetylation of **L** affords *aspirin* **M**.

Moreover, **L** is a precursor of a dye *Aluminon* used for quantitative determination of aluminum and some other metals. Reaction of two equivalents of **L** with formaldehyde under acidic conditions affords **N**. Addition of one more equivalent of **L** to **N** in the presence of $NaNO_2$ and sulfuric acid yields **O**, which finally gives *Aluminon* upon treatment with ammonia.



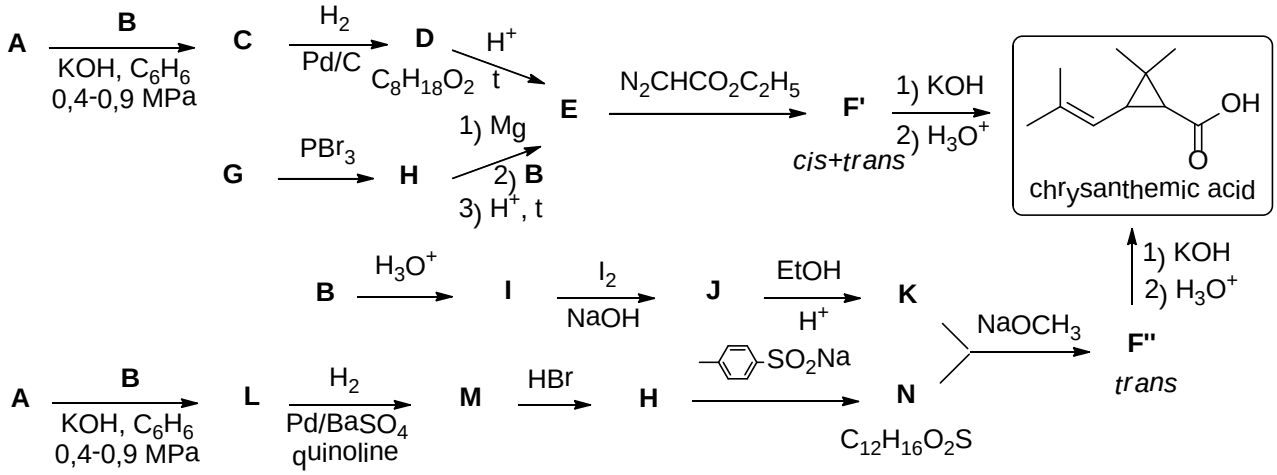
1. Write down the structural formulae of **A-E** and **G-O**.

2. Write down the structure of monomeric unit **F**.

Problem 19. Chrysanthemic acid

Insecticides are substances preventing us from insects by destroying, repelling or mitigating them. The use of insecticides is one of the major factors behind the increase in agricultural productivity in the 20th century. Insecticides are also used in medicine, industry and housekeeping. Natural insecticides, such as nicotine and esters of chrysanthemic acid, are produced in plants. On the contrary to nicotine, esters of chrysanthemic acid are non-toxic to man and other mammals.

Many methods for chrysanthemic acid synthesis have been described to date. Two of these are presented in the hereunder scheme (the first step of both methods is the reaction discovered in 1905 by the Russian chemist A. Favorskii).

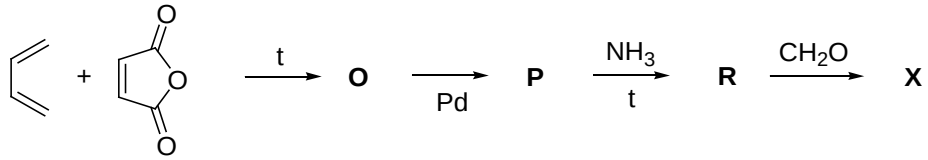


1. Write down the structural formulae of all compounds given in this scheme. Note that **A** is a gaseous hydrocarbon with the density lower than that of air, **G** is a natural alcohol, **F'** is a mixture of isomers, whereas **F''** is formed only in trans-form.

Methods given in the scheme provide chrysanthemic acid as a mixture of stereoisomers, while natural chrysanthemic acid has (1R,3R)-configuration.

2. Write down the structural formulae of natural chrysanthemic acid.

Tetramethrin is a key substance of many household insecticides. This compound belonging to pyrethroids of the 1st generation can be obtained by esterification of chrysanthemic acid with alcohol **X**. Synthesis of the latter is given below.



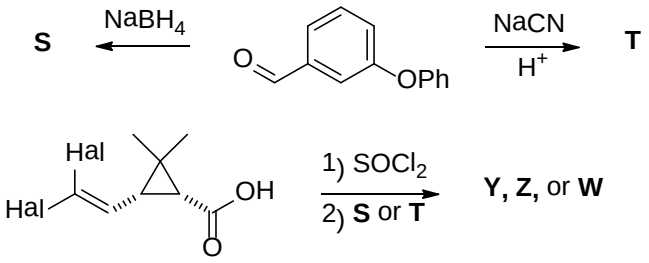
3. Write down the structural formulae of **O**-**R**, and **X**. Note that the transformation of **O** into **P** is an isomerization with retention of the carbocyclic skeleton leading to the most stable isomer.

Synthesis of Tetramethrin is completed by the reaction of **X** with chrysanthemic acid or some of its derivatives.

4. Which of the following acid derivatives could easily form esters in reaction with alcohols?

a) anhydride; b) methyl ester; c) amide; d) hydrazide

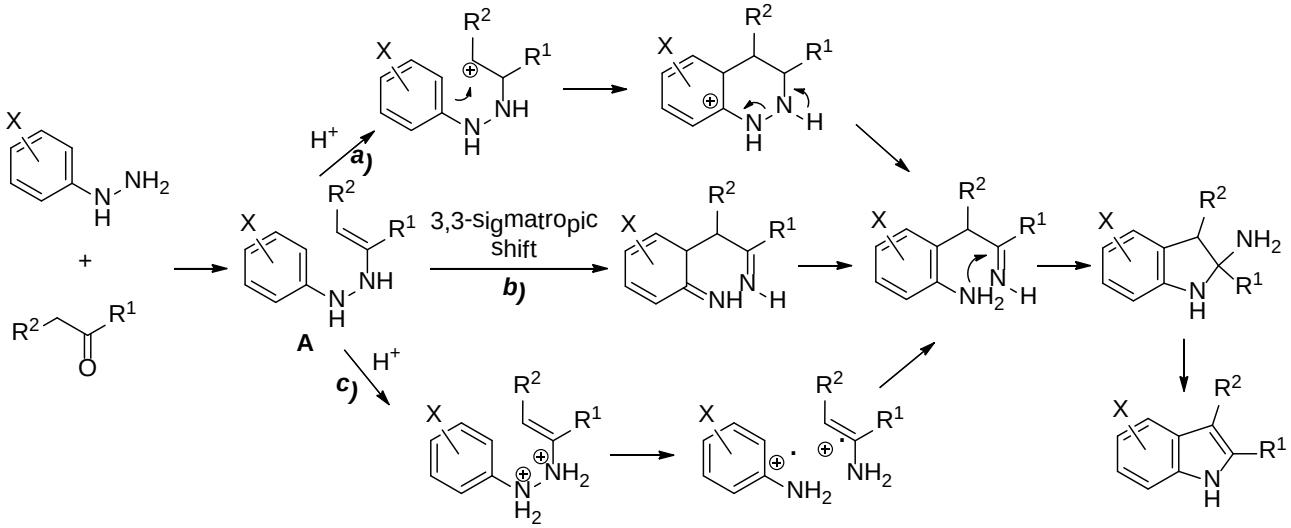
The 1st generation pyrethroids are photochemically unstable, which stimulated development of new types of pyrethroids (of the 2nd and 3rd generations). In particular, substitution of the $CH=C(CH_3)_2$ fragment in chrysanthemic acid by the $CH=CHal_2$ moiety increases photostability of pyrethroids. Thus, three compounds (*cis*-permethrin, **Y**, cypermethrin, **Z**, and deltamethrin, **W**) were prepared from *cis*-2-(2,2-dihalovinyl)-3,3-dimethylcyclopropane-1-carboxylic acid and 3-phenoxybenzaldehyde according to the scheme below.



5. Write down the structural formulae of **S**, **T**, **W**, **Y**, **Z**. Note that the halide content in **W**, **Y**, **Z** is 31.6, 18.1, and 17.0 %, respectively.

Problem 20. Heterocycles

Chemists are fascinated with pyrroles and their benzannulated derivatives, indoles, for more than 150 years owing to the high diversity of their transformations and a broad spectrum of bioactivity. Fischer synthesis starting from arylhydrazines and ketones is the classical method providing for various indoles. For a long time, the mechanism of this reaction was under discussion, and three pathways given below were considered as alternatives.



1. Write down the mechanism of enhydrazine **A** formation.

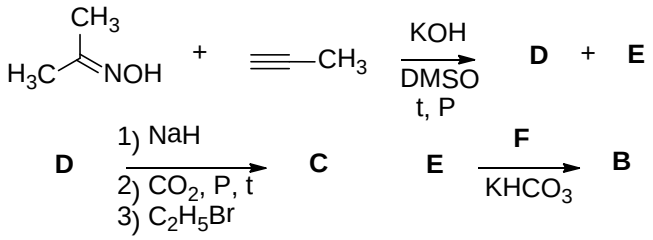
In 1970s, the Russian scientist I. Grandberg investigated a reaction of *N*,*N*-diarylhydrazines $Ar^1Ar^2NNH_2$ with ketones and discovered that a mixture of two indoles in a ratio of *ca.* 1:1 is formed, the result being independent of the substituent nature (donor or acceptor) in the aryl groups. These experiments proved unambiguously the mechanism of the Fischer indole synthesis.

2. Point out the mechanism (***a, b*** or ***c***) proved by I. Grandberg.

The Paal-Knorr reaction of amines with 1,4-diketones is the classical synthesis of a pyrrole core. Still, some amines can form the pyrrole ring in the reaction with 1,3-diketones. Thus, ethyl ester of glycine (aminoacetic acid) provides pyrrole derivatives **B** and **C** in an acid-catalyzed reaction with hexane-2,5-dione and a base-catalyzed reaction with pentane-2,4-dione, respectively.

3. Write down the structural formulae of **B** and **C**.

The Russian chemist B. Trofimov with collaborators developed a method of pyrrole synthesis from oximes and alkynes. Thus, treatment of a mixture of acetone oxime and propyne with KOH in DMSO under heating produced pyrroles **D** and **E**.

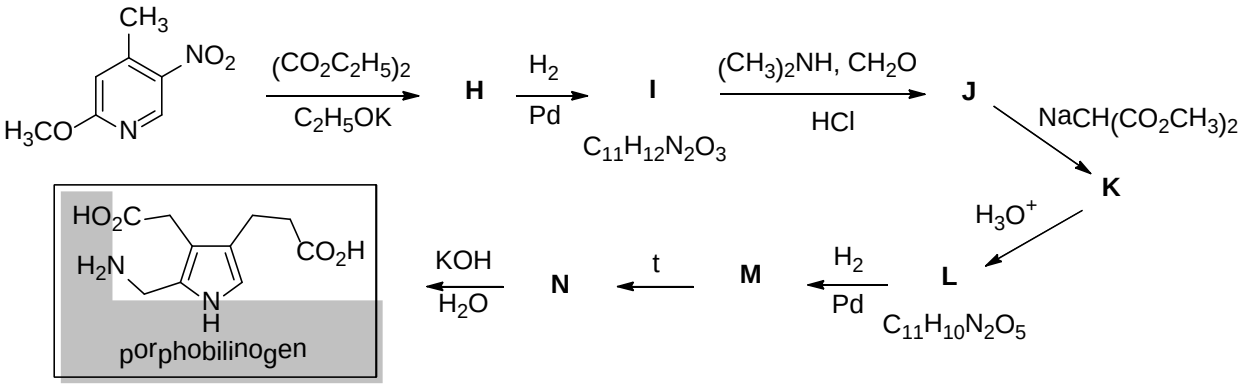


4. Write down the structural formulae of **D**-**F**. Note that the carbon content in **F** is 28.7%.

Use of alkynes with electron-withdrawing groups allows applying milder reaction conditions. Thus, acetophenone oxime reacts with ethyl propynoate affording a single product **G** upon treatment with 4-(dimethylamino)pyridine in toluene under microwave irradiation.

5. Write down the structural formula of **G**.

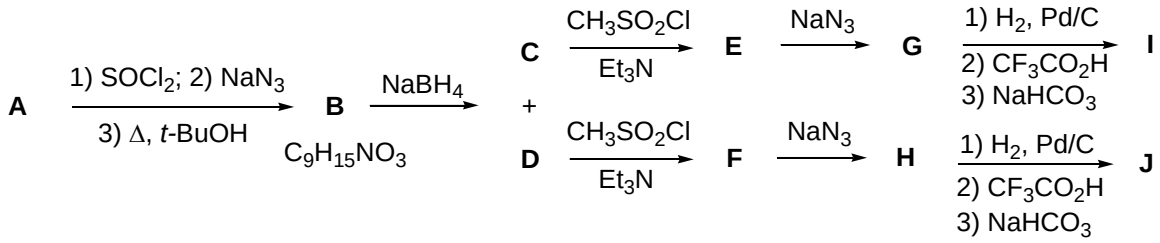
Pyrrole ring is a key moiety of many bioactive natural compounds including porphobilinogen, an intermediate in biosynthesis of heme and chlorophyll. This compound was synthesized in laboratory according to the hereunder scheme.



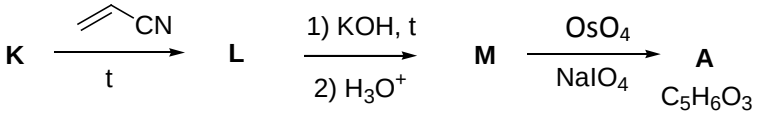
6. Decipher the scheme and write down structural formulae of **H**-**N**.

Problem 21. Cyclobutanes

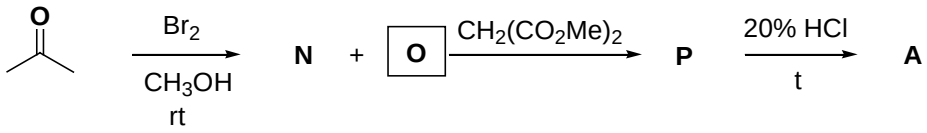
In 1894 Emil Fischer proposed the “lock and key” principle for interaction between a drug and its molecular target. The interaction is efficient only in case of substances having specific complementary geometry that fit exactly to the molecular target. According to this model, a potential drug should accept a definite conformation with the appropriately located functional groups. One of ways to achieve this goal is restriction of conformational mobility of molecules. Recently Ukrainian chemists reported synthesis of conformationally rigid diamines **I** and **J** according to the scheme below.



The starting compound **A** was synthesized for the first time in 1958 by J.D. Roberts and F.F. Caserio (authors of the classical textbook on organic chemistry), according to the scheme:



Another method for **A** synthesis is given below:



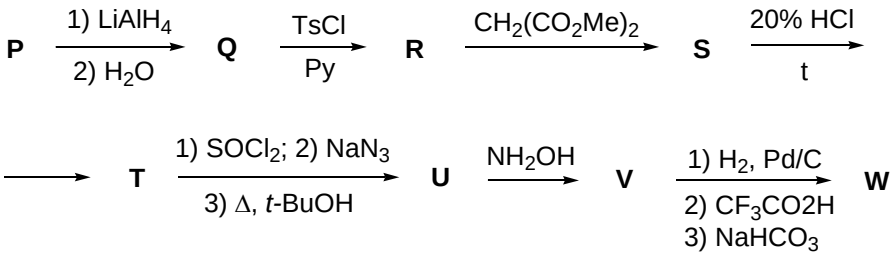
1. Decipher the schemes. Write down the structural formulae of compounds **A**-**P** accounting for the following:

a) **C** and **D** are isomers; **J** has two planes of symmetry;

b) hydrocarbon **K** has a single type of hydrogen atoms; ω_H = 10.0%;

c) **N** and **O** are isomers; ω_H = 3.8%; ω_C = 22.9%.

Starting from **P**, a very interesting compound **W** was synthesized:



2. Write down the structural formulae of **Q**-**W**.

3. Can **W** be resolved into enantiomers?

Problem 22. Introduction to translation

Biosynthesis of proteins, also known as translation, proceeds at ribosomes found to be large multi-component supramolecular complexes composed of ribosomal RNA and proteins. The first stage of translation (referred to as initiation) includes assembling of large and small ribosomal subparticles together with messenger RNA (mRNA) as it is shown in Fig. 1.

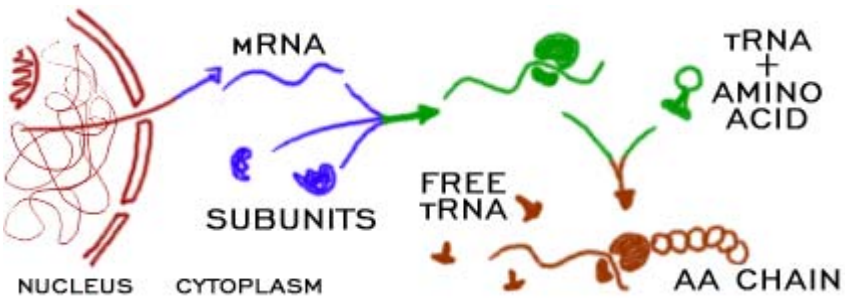


Figure 1. General scheme of protein translation in a living cell (http://www.biology4kids.com/files/cell_ribos.html)

1. Any amino acid is encoded by a codon, a sequence of three nucleotide residues in mRNA. How many codons do exist, if only four main ribonucleotides are taken into consideration? Do all codons encode amino acids?

2. Is it possible to derive a unique ribonucleotide sequence for a protein with a known amino acid sequence?

Amino acids are delivered to a functioning ribosome by a specific small RNA (referred to as transfer RNA, or tRNA). Each tRNA corresponds to a sole codon.

3. How many different tRNAs can deliver an individual amino acid to ribosome? Consider leucine and methionine.

To be delivered to a ribosome, an amino acid should be covalently bound to its tRNA. This reaction requires energy provided by ATP hydrolysis and is catalyzed by aminoacyl-tRNA synthetase (aaRS), an enzyme specific for a particular amino acid. The side chain of the attached amino acid is not involved in covalent linkage with the tRNA.

4. Write down equation(s) of the reaction(s) catalyzed by aaRS during the process of amino acid binding to tRNA. Indicate groups of the tRNA and amino acid involved in the linkage formation.

5. Using the table of genetic code write down amino acid sequences for the oligopeptides:
a) encoded by the hereunder mRNA
b) encoded by the hereunder mRNA with the first and the last C replaced by U
c) encoded by the hereunder mRNA with the first G replaced by C
d) encoded by the hereunder mRNA with the last but one G replaced by U

5'AUGGAUCACGCCAUCAAUGUUGUCGGUUGGAGUGUGGAUACGUUGGAUGAUGG
AACUGAAGCU3'.

6. Write down the nucleotide sequence of mRNA encoding the peptide Met-Asp-Val-Asn-His-Pro-Glu-Tyr-Gly-Lys. Use A, U, G, and C for unambiguously decided positions, **N1/N2** if any of two nucleotides is possible at a particular position, and **N** if any of four nucleotides is possible at a particular position (**N1** and **N2** can be any of A, U, G, and C).

7. Molecular weight of an *E.coli* protein is of about 51 kDa. Estimate the length of encoding mRNA (in nm, rounding to integer). Take the average molecular weight of an amino acid as 110 g/mol, and the average length of a ribonucleotide residue as 0,34 nm. How long will it take a cell to synthesize this protein if the ribosome reads 20 ribonucleotide residues per second?

A group of researches accomplished protein synthesis in a cell-free system (*in vitro*). All required components (ribosomes, tRNAs, ATP, GTP, salts, amino acids, aaRS, translation factors, etc.) were added to the system. A synthetic polyribonucleotide consisting of only A and C in the ration of 1:5 was used as the messenger RNA (nucleotide residues are arranged randomly in the mRNA).

8. Determine the amino acid composition of the synthesized protein. What are the ratios between the amino acid residues in the protein?

The 3D structure of a tRNA is depicted in Fig. 2. There are two key regions: the CCA3' terminus which is linked to the amino acid, and the anticodon exactly matching to the mRNA codon.

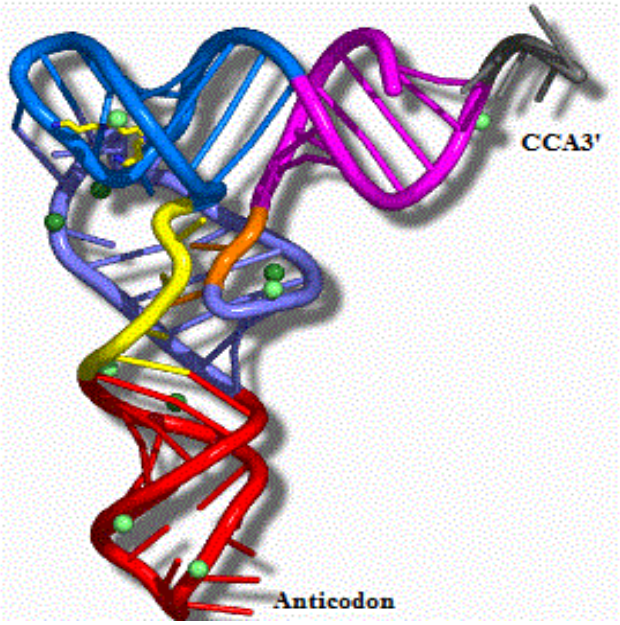


Fig. 2. The 3D structure of a tRNA

9. A mutant $tRNA^{Tyr}$ with anticodon specific to Ser codon (instead of Tyr codon) was introduced into the synthetic system described in i.8. What would be the resultant protein?

A biochemist specializing in protein chemistry described his discovery of a new mutant protein with Glu to His mutation to a molecular geneticist. The latter was very much surprised and advised the biochemist to do a double check.

10. Why did the geneticist express a doubt concerning the possibility of the above mutation? What mutation is more probable?

Problem 23. Intriguing translation

Borrow trouble for yourself, if that's your nature, but don't lend it to your neighbours
Joseph Rudyard Kipling

An acyclic oligopeptide **X** is composed of residues of two proteinogenic (canonical, encoded) amino acids **A** and **B**. The prevalent ionic form of **X** in aqueous solution at pH 4.7 consists of 25 atoms.

1. Determine the number of amino acid residues in **X**. Use the information provided by the Wikipedia at either http://en.wikipedia.org/wiki/Proteinogenic_amino_acid or http://en.wikipedia.org/wiki/Amino_acid (hint: pay attention to the given pK_a values of amino acid side groups).

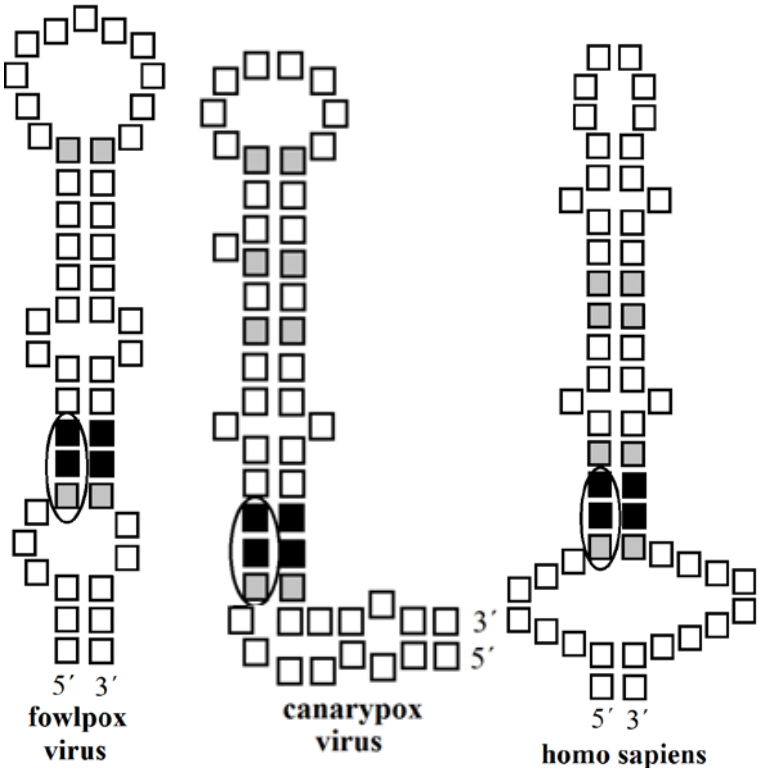
2. How many individual peptides are in agreement with the above information?

Combustion of 1.000 g of **X** in an excess of oxygen followed by absorption of the reaction products with an excess of calcium hydroxide solution leads to formation of 3.273 g of precipitate. Quantitative transfer of the filtered precipitate into 10% aqueous hydrochloric acid results in liberation of 0.496 L of gas (STP – standard temperature and pressure).

3. Draw the stereochemical structure of **X** supporting it by appropriate calculations. Specify the absolute configuration (R or S) of chiral centers in **X**.

4. Explain why **A**, in contrast to **B**, is not found as a free amino acid in living cells.

Addition of amino acid **A** to a growing polypeptide chain during translation is possible only in case of a certain motive (**Element X**) in the secondary structure of messenger ribonucleic acid (mRNA). Element **X** is a hairpin with two loops composed of approximately 60 nucleotides. Three such motives determining synthesis of glutathione peroxidase fragments in different organisms are schematically given hereunder (left to right: *Poxviridae* host cell infected with fowlpox, *Poxviridae* host cell infected with canarypox virus, and human cell).



Each square box in the pictures stands for a nucleotide residue with one of the canonical nitrogen bases: adenine (A), guanine (G), uracil (U) or cytosine (C). Hydrogen bonds are formed according to the complementary principle (Chargaff's rule) between the bases with boxes opposite to each other. The only exceptions are:

- Nucleotides with boxes filled grey: pairs are formed by either two pyrimidines or these are unusual pairs A-C or G-U
- Nucleotides with boxes filled black: pairs are formed by two purines
- Nucleotides located in the middle of the upper loops and visually close to each other due to way of the hairpins representation.

The mRNA triplet (codon) identical for all three sequences is circled.

Fragments of mRNA sequences belonging to different organisms are given in the hereunder Table in an arbitrary order. These sequences contain **Elements X** depicted in the above images.

№	Nucleotide sequence (5′→3′)
1	…GCUGCUAAUGAAGAAAUGACUAUAAAUAGAUGGGUCAUGCCUGACACGCAAAG…
2	..AGGCACUCAUGACGGCCUGCCUGCAAACCUGCUGGUGGGGCAGACCCGAAAAUCCCAC…
3	…GACGAGAUAAUGAAGAAAUGGUCCUAAACAGAUGGGUCGUUCCUGACACCCCGG…

5. Fill the boxes in the images of all three structures, using one-letter symbols for nucleotides, and correlate the images with fragments of mRNA. Note that the sequences in the Table are bit longer than fragments corresponding to Elements X.

6. Draw the unusual base pair guanine-uracil found in the hairpin structure, and show the hydrogen bonds.

7. What is the role of the encircled codon in the case of poxoviruses (but not humans!)? Note that the subsequent triplet determines inclusion of the next amino acid into the growing polypeptide chain. Choose only one answer.

№	Answer
1	It interacts with transport RNA of amino acid **A**
2	It determines termination of biosynthesis of the viral polypeptides on ribosome
3	It forms a *"foot"* of the lower loop thus playing a purely structural role
4	It is unable to interact with aminoacyl-tRNA. Thus the ribosome ignores it continuing addition of amino acids from the next codon
5	It is an ordinary codon without any special features

RNA-containing viruses are characterized by frequent mutations allowing better adaption to changing environmental conditions.

8. For each of viral sequences, propose a mutation (single nucleotide substitution by another one), which presumably would not affect either translation or glutathione peroxidase functioning. Use the table of codons at http://en.wikipedia.org/wiki/Genetic_code.

Problem 24. Unusual amino acids: search for new properties

If you want it to be done, do yourself
Mr. Zorg, "Fifth Element"

Search for natural compounds with anti-cancer potential is one of rapidly developing branches of modern science. Results of a recent research will be considered below.

X is a potential antineoplastic drug. In order to study mechanisms of its formation from different precursors, a mixture of three synthesized in the laboratory compounds **A**, **B** and **C** was administered orally to rats at doses of 63.5, 58.5 and 39.6 μg per kg of body weight, respectively.

A and **B** are stable α-amino acids found in nature. Residue of one of these compounds is detected in proteins. Information about **A**, **B**, and **C** is summed up in the table below:

Compound	Content, mass %			Number of elements forming the compound	Number of chiral atoms
	C	H	O		
A	31.09	5.74	16.57	5	1
B	26.67	5.04	17.77	5	1
C	9.24	3.10	Is found in **C**	4	0

It is also known that**:**

- **A**, **B** and **C** have molecular weight of less than 250 g/mole each;
- **A**, **B** and **C** contain C, H, N and O (not obligatory all these elements) in usual (native) isotopic ratios;
- The number of nitrogen atoms obeys the following inequality: N_{nitrogen} **(B)**$\geq N_{\text{nitrogen}}$**(A)**.

1. Considering all possibilities for the number of nitrogen atoms in **A** and **B**, determine their elemental composition.

2. If you failed to get the answer in i. 1, take advantage of an additional hint: **A** and **B** contain the same number of nitrogen atoms.

3. Draw all possible structures of **B** (without stereochemical details).

4. If the provided data is sufficient, indicate the absolute configuration (R or S) at the stereocenters of the structures in i.3.

During the experiment, samples of air exhaled by test animals were collected at definite time intervals. The following substances (in addition to other metabolites) were detected:

Detected gaseous compound	Density rel. H_2	Precursor compound
A1	53	**A**
B1	53.5	**B**
C1	56	**C**

5. Draw the structures of **A1** and **B1**, if it is known that **A1** has only identical atoms of hydrogen and does not contain π-bonds.

Formation of **C1** from **C** in rats proceeds via two enzymatic stages: reduction of **C** giving intermediate **X** is followed by its transformation into **C1**.

6. Determine the structures of **C**, **C1**, and antineoplastic metabolite **X**, if it is known that **C** does not contain C–O bonds.

Formation of **A1** and **B1** from **A** and **B**, respectively, also occurs in two steps, the latter being catalyzed by the same enzyme as was involved in transformation of **X** into **C1**.

7. Determine the structures of **A** and **B**.

8. Comment on the choice of **A**, **B**, and **C** masses in the mixture administered to rats.

One of the amino acids discussed above can be found in proteins. It is also know that this amino acid does not have its own transfer RNA (tRNA).

9. Decide the residue of which amino acid (**A** or **B**) can be found in proteins. From the variants listed below, choose one explaining how it appears in proteins.

№	Variant
1	**A**, because it is formed as a result of the one-step post-translational modification of a canonical amino acid
2	**A**, because it is structurally similar to a canonical amino acid, which sometimes leads to false insertion during translation
3	**A**, because it can be involved in protein biosynthesis at ribosomes without pre-formation of aminoacyl-tRNA
4	**B**, because it is structurally similar to a canonical amino acid, which sometimes leads to false insertion during translation
5	**B**, because it can be involved in protein biosynthesis at ribosomes without pre-formation of aminoacyl-tRNA

Problem 25. Specific features of *Clostridium* metabolism

Imagination is more important than knowledge
Albert Einstein

As first shown in 1993, a type of acidogenic (producing acid) *Clostridium* bacteria is capable of glucose fermentation at certain conditions according to the hereunder total reaction equation:

$$5C_6H_{12}O_6 + kH_2O \rightarrow l\mathbf{A} + m\mathbf{B} + n\mathbf{C} + 10\mathbf{D} \qquad (1),$$

where *k, l, m, n* are integers.

A and **B** are unbranched saturated carboxylic acids, **C** and **D** are gases (at STP) free of C–H bonds. The obtained mixture of **C** and **D** has the density rel. H_2 of 10.55.

1. Draw the structural formulae of **C** and **D**.

2. Mathematically prove that each of **A** and **B** is a monocarboxylic acid.

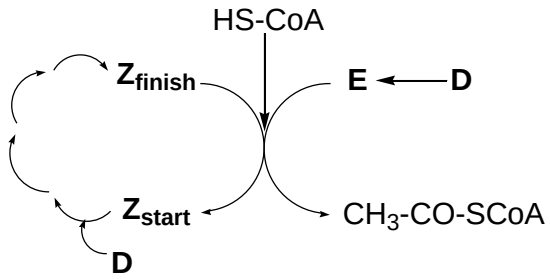
3. Choose the appropriate l:m ratio for the reaction (1) from the variants given below.

Variant	*l*:*m* ratios
a.	1:1
b.	1:2
c.	1:3
d.	1:4
e.	1:5
f.	Other ratio

Note that the fermentation products contain less carbon atoms than the starting compound.

4. Draw all possible variants of **A** and **B**.

Clostridium is capable of utilizing **D** in an unusual synthesis of acetyl-CoA (coenzyme A). This synthetic process is conjugated with cyclic metabolism of a vitamin derivative **Z** according to the following scheme:



$\mathbf{Z_{start}}$ and $\mathbf{Z_{finish}}$ contain the same number of nitrogen atoms. Molar fractions (χ) of nitrogen and hydrogen are given below:

Compound	χ (H),%	χ (N),%
$\mathbf{Z_{start}}$	43.103	12.069
$\mathbf{Z_{finish}}$	41.818	12.727

5. Determine the total number of atoms in $\mathbf{Z_{start}}$ and $\mathbf{Z_{finish}}$, if it is known that these are less than 100 for both compounds.

Back in 1952, it was shown that cultivation of *Clostridium thermoaceticum* under anaerobic conditions in the presence of only non-radioactive **D** isotopologues (compounds **D1** and **D2**) gives rise to formation of acetyl-CoA isotopologues with the equal mass fraction of N (12.08 %). Moreover, no traces of unlabeled acetyl-CoA (*M*=809.6 g/mol) were detected in the experiment.

6. Work out the formulae of **D1**, **D2**, and **E**, if all the coefficients in the reaction equation of acetyl-CoA formation are equal to 1.

Study of Clostridium transcriptome revealed a short (~100 nucleotides) coding sequence composed of only guanine (G) and cytosine (C) present in equimolar quantities and randomly positioned.

7. What is the ratio between the amino acid residues in the olygopeptide encoded by the sequence? Choose only one correct variant.

Variant	Ratio	Variant	Ratio
1	1:1:2	4	1:1:4:2
2	1:1:3	5	1:2:2:2:1
3	1:1:1:1	6	Data insufficient to choose a sole variant

One of the proteins synthesized by *Clostridium* consists of 238 amino acid residues. Positions 230 to 234 (from *N*-terminus) were identified as Trp-His-Met-Glu-Tyr. A mutation affecting only one nucleotide occurred in the gene region corresponding to the above peptide fragment. As a result, the length of biosynthesized protein decreased up to 234 amino acid residues, whereas the sequence in positions 230 to 234 changed to Trp-Thr-Tyr-Gly-Val.

8. Write down the only possible original (before mutation) mRNA sequence encoding the above peptide fragment.

Problem 26. Analysis of complex formation

Antibodies **Ab** are proteins capable of selective binding with specific antigen **Ag** species (usually protein or polysaccharide), thus forming the so-called immune complex **Ab*Ag**. The binding constant of the process K_b is very high (around 10^9), however, binding is reversible.

$$\mathbf{Ab} + \mathbf{Ag} \rightleftarrows \mathbf{Ab} * \mathbf{Ag}$$

Despite of seeming complexity of biological objects, their functional features can often be analyzed by simply treating **Ag** and **Ab** as a ligand and complexing agent, respectively, in a common reaction of the **Ab*Ag** complex formation. Moreover, specific binding of proteins with other ligands (enzyme inhibitors, lipids, methal ions, etc.) can be analyzed by using the same approach.

1. Express K_b as a function of equilibrium concentrations [Ab], [Ag], [Ab*Ag] (consider that 1:1 Ab*Ag complex is formed).

Parameter $\bar{\mathbf{n}}$ is an average number of Ag molecules bound to one Ab molecule. In the case of only one binding site in Ab, $\mathbf{0} \leq \bar{\mathbf{n}} \leq \mathbf{1}$.

2. Express $\bar{\mathbf{n}}$ as a function of K_b and equilibrium concentration of the unbound ligand [Ag] for this simplest case of a single binding site in Ab molecule. Assume that K_b remains unchanged in course of the binding process. Draw schematically the $\bar{\mathbf{n}}$ vs [Ag] plot ("titration" curve of Ab with Ag).

For easier and reliable analysis, the titration curve may be linearized in special coordinates.

3. a) Plot the Experimental data A (see the table below) as **[Ab*Ag]/[Ag]** *vs* **[Ab*Ag]**.

b) Express **[Ab*Ag]/[Ag]** as a function of **[Ab*Ag]**.

c) One of the data points in the Experimental data A set has been determined incorrectly. Encircle this outlier in the plot.

d) Suggest a way for K_b determination from the plot analysis.

e) In the same plot, draw schematically a curve for ADP binding with another ligand, if the latter is characterized by a 10 times higher K_b value (as compared to that for **ADP*Mg**$^{2+}$ complex formation).

Experimental data set A

ADP protein binds with Mg^{2+} in 1:1 complex (single binding site, one Mg^{2+} per site). K_b is not dependent on $\bar{n}$. ADP total concentration is kept constant at 80 μM.

Mg^{2+} total concentration, μM	Bound Mg^{2+} concentration, μM
20.0	11.6
50.0	26.0
100	42.7
150	52.8
200	59.0
300	61.1
400	69.5

Some antibodies can only bind a single antigen molecule, whereas others bind two (or even more) antigen molecules. Maximal number of **Ag** molecules that can be bound to a single **Ab** is referred to as the **Ab** valence.

4. a) Derive an expression to be used for determination of the **Ab** valence from the plot analysis in coordinates **[Ab*Ag]/[Ag]***vs* **[Ab*Ag]**.

b) Plot the Experimental data B using the above coordinates. Determine the enzyme valence.

Experimental data set B

An enzyme binds with its inhibitor **I**, the binding to different sites is independent, and K_b is the same. Enzyme total concentration is kept constant at 11 μM.

I total concentration, μM	Free (unbound) **I** concentration, μM
5.2	2.3
10.4	4.95
15.6	7.95
20.8	11.3
31.2	18.9
41.6	27.4
62.4	45.8

Ab specimen often contains admixtures of other proteins not capable of binding with **Ag**. Thus, a "known" total **Ab** concentration includes both functionally active antibodies and unreactive proteins.

5. a) Suggest a way for determination of the actual **Ab** concentration from the data analysis in coordinates **[Ab*Ag]/[Ag]***vs* **[Ab*Ag]**.

b) Does the ADP specimen contain any unreactive admixtures (Experimental data A)?

c) Why is it impossible to conclude unambiguously about the presence of unreactive admixtures in the enzyme specimen (Experimental data B)? What helpful (to determine the admixtures concentration) data is missing?

Problem 27. Inorganic polymers: polyphosphates and polysilicones

There are few elements capable of forming elementary substances with long-chain molecules.

1. Give 3 examples of elements, atoms of which can form elementary substance with linear (or close to linear) chain molecules (longer than 10 atoms).

Such long-chain elementary substances are not very common. However, many elements can form heteroatomic long-chain molecules. High-polymeric inorganic polyphosphates can serve as an example. These compounds are linear polymers composed of orthophosphate residues. The condensation reaction is one of the ways of such polymer formation.

2. Write down the condensation reaction giving diphosphate from the orthophosphate precursor.

3. In general, condensation reactions are reversible. Write down the equilibrium constant of the condensation reaction between phosphate oligomers, provided that polyphosphate species of different polymerization degree (including monomers) are not kinetically distinguishable. Assume that each (poly)phosphate ion present in the system bears only a single bound proton (i.e. may be represented as $P_iO_{3i}OH^{(i+1)-}$).

4. Of the synthetic routes to long-chain polyphosphoric acids listed below, choose the most and the least energetically favorable. Take into account that the P–O bond is macroergic (for instance, $\Delta G^{\circ'}$ of adenosine triphosphate hydrolysis into adenosine diphosphate and inorganic phosphate is of about –31 kJ/mol).

i) H_3PO_4 condensation in 1 M aqueous solution at room temperature.
ii) H_3PO_4 condensation in concentrated solution at room temperature.
iii) H_3PO_4 condensation with dichlorophosphoric acid HPO_2Cl_2 at elevated temperature.

In many cases, the equilibrium constant of a condensation reaction is too low to provide for high-molecular weight products. Other condensation reactions are too fast, which results in complexity of their control. To overcome these drawbacks, a procedure to form condensing species *in situ* from corresponding precursor has been developed.

5. Draw the structural formulae of isomeric compounds $C_2Cl_3H_5Si$ if none of these contains Si–H bonds. Write down a scheme of condensation of these compounds (in the presence of water) yielding a long-chain molecule. What are the atoms forming the main chain of the product?

6. Which of the isomeric compounds $C_2Cl_3H_5Si$ from i. 5 gives the linear condensation product only? Draw the structure of the final condensation product provided all the reactions are by 100% complete. What functional groups may be additionally found in the product due to incomplete hydration or condensation reactions?

7. Write down a reaction scheme illustrating appearance of branching in the main chain during condensation of another isomeric compound $C_2Cl_3H_5Si$ from i. 5 (that not chosen in i. 6).

THE SAFETY RULES AND REGULATIONS

Regulations of the International Chemistry Olympiad (IChO)

§ 12 Safety

(1) During the experimental part, the competitors must wear laboratory coats and eye protection. The competitors are expected to bring their own laboratory coats. Other means of protection for laboratory work are provided by the organizer.

(2) When handling liquids, each student must be provided with a pipette ball or filler. Pipetting by mouth is strictly forbidden.

(3) The use of very toxic substances (designation T+) is strictly forbidden. The use of toxic substances (designation T) is not recommended, but may be allowed if special precautions are taken. Substances belonging to the categories R 45, R 46, R 47 must not be used under any circumstances (see Appendix B for definitions of these categories).

(4) Detailed recommendations involving students´ safety and the handling and disposal of chemicals can be found in Appendices A 1, A 2, and B. These appendices are based on the directives of the European Communities and are updated automatically with these directives.
a) Appendix A 1: Safety Rules for Students in the laboratory.
b) Appendix A 2: Safety Rules and Recommendations for the Host Country of the IChO.
c) Appendix B contains:
B 1: Hazard Warning Symbols and Hazard Designations;
B 2: R-Ratings and S-Provisions: Nature of special risks (R) and safety advice (S);
B 3: Explanation of Danger Symbols (for use of chemicals in schools).

APPENDIX A

A 1: SAFETY RULES FOR STUDENTS IN THE LABORATORY

All students of chemistry must recognize that hazardous materials cannot be completely avoided. Chemists must learn to handle all materials in an appropriate fashion. While it is not expected that all students participating in the International Chemistry Olympiad know the hazards of every chemical, the organizers of the competition will assume that all participating students know the basic safety procedures. For example, the organizers will assume that students know that eating, drinking or smoking in the laboratory or tasting a chemical is strictly forbidden.

In addition to the common-sense safety considerations to which students should have been previously exposed, some specific rules, listed below, must also be followed during the Olympiad. If any question arises concerning safety procedures during the practical exam, the student should not hesitate to ask the nearest supervisor for direction.

Rules regarding personal protection

1. Eye protection must be worn in the laboratories at all times. If the student wears contact lenses, full protection goggles must also be worn. Eye protection will be provided by the host country.

2. A laboratory coat is required. Each student will supply this item for himself/herself.

3. Long pants and closed-toed shoes are recommended for individual safety. Long hair and loose clothing should be confined.

4. Pipetting by mouth is strictly forbidden. Each student must be provided with a pipette bulb or pipette filler.

Rules for Handling Materials

1. Specific instructions for handling hazardous materials will be included by the host country in the procedures of the practical exam. All potentially dangerous materials will be labeled using the international symbols below. Each student is responsible for recognizing these symbols and knowing their meaning (see Appendix B 1, B 2 and B 3).

2. Do not indiscriminately dispose chemicals in the sink. Follow all disposal rules provided by the host country.

A 2: SAFETY RULES AND RECOMMENDATIONS FOR THE HOST COUNTRY OF THE INTERNATIONAL CHEMISTRY OLYMPIAD

Certainly it can be assumed that all students participating in the IChO have at least modest experience with safety laboratory procedures. However, it is the responsibility of the International Jury and the organizing country to be sure that the welfare of the students is carefully considered. Reference to the Safety Rules for Students in the Laboratory will show that the students carry some of the burden for their own safety. Other safety matters will vary from year to year, depending on practical tasks. The organizers of these tasks for the host country are therefore assigned responsibility in the areas listed below. The organizers are advised to carefully test the practical tasks in advance to ensure the safety of the experiments. This can best be accomplished by having students of ability similar to that of IChO participants carry out the testing.

Rules for the Host Country (see also A 1):

1. Emergency first-aid treatment should be available during the practical examination.

2. Students must be informed about the proper methods of handling hazardous materials.

a) Specific techniques for handling each hazardous substance should be included in the written instructions of the practical examination.

b) All bottles (containers) containing hazardous substances must be appropriately labeled using internationally recognized symbols (see Appendix B 1).

3. Chemical disposal instructions should be provided to the students within the written instructions of the practical examination. Waste collection containers should be used for the chemicals considered hazardous to the environment.

4. The practical tasks should be designed for appropriate (in other words, minimum) quantities of materials.

5. The laboratory facilities should be chosen with the following in mind:

a) Each student should not only have adequate space in which to work, but should be in safe distance from other students.
b) There should be adequate ventilation in the rooms and a sufficient number of hoods when needed.
c) There should be more than one emergency exit for each room.
d) Fire extinguishers should be near by.
e) Electrical equipment should be situated in an appropriate spot and be of a safe nature.
f) There should be appropriate equipment available for clean-up of spills.

6. It is recommended that one supervisor be available for every four students in the laboratory to adequately ensure safe conditions.

7. The organizers should follow international guidelines for the use of toxic, hazardous or carcinogenic substances in the IChO.

APPENDIX B

B 1: HAZARD WARNING SYMBOLS AND HAZARD DESIGNATIONS AND THEIR EXPLANATION (Applied for Chemicals in Schools)

1. Explosive substances (E)

These are substances which can be caused to explode by exposure to a flame or which are more sensitive to impact of friction than 1,3-dinitrobenzene (e.g. picrates, organic peroxides). In particular they include substances with R ratings R1 - R3 (see B 2), designation E.

When using and storing these substances, the S provisions (S15 - S17) must be observed (see B 2).

2. Fire inducing substances, Oxidizing (O)

These are substances which can have a strong exothermic reaction on coming into contact with other, particularly flammable substances or organic peroxides. They include in particular substances R 7 to R 9, designation O.

3. Highly flammable, easily flammable and flammable substances (F+, F)

In liquid form, highly flammable substances have an ignition point below 0 °C and a boiling point of 35 °C maximum. They are to be designated by the danger symbol F+ and the rating R 12.

Substances are easily flammable if they:
a) can heat up and ignite at normal air temperature without energy supply,
b) are easily ignited in solid state by short exposure to a source of flammation and continue to burn or glow after removal of the latter,
c) ignite below 21 °C in liquid state,
d) ignite in gaseous state if mixed with air at 101.3 kPa and 20 °C,
e) develop easily flammable gases in dangerous quantities when in contact with water or damp air,
f) ignite if brought into contact with air when in dust-like state.

These substances are to be designated with the danger symbol F and the rating R 11.

Flammable substances have in liquid form an ignition point of 21 °C to 55 °C and are to designated with the rating R 10, no danger symbol.

When dealing with highly flammable, easily flammable and flammable liquids may only be heated using sealed electrical heating equipment which is not in itself a source of flammation. All substances must be heated in such a way that the dangerous vapors liberated by heating cannot escape into the atmosphere. This does not apply to fire hazardous substances in small quantities for fire demonstrations.

The regulations laid down by the state fire authorities must be observed.

4. Toxic substances (T +, T, Xn)

Legislation applying to chemicals distinguishes three categories of toxicants:
highly toxic substances (R 26 R 28), danger symbol T+,
toxic substances (R 23 R 25), danger symbol T,
less toxic substances (R 20 R 22), danger symbol Xn.

Highly toxic substances are those which can cause grave acute or chronic health damage or death almost immediately if inhaled, swallowed or absorbed through the skin in small amounts.

Toxic substances are those which can cause considerable acute or chronic health damage or death if inhaled, swallowed or absorbed through the skin in small amounts.

Less toxic substances (noxious substances) are those which can cause restricted health damage if inhaled, swallowed or absorbed through the skin.

If highly toxic or toxic substances are produced in the course of an experiment (e.g. chlorine, hydrogen sulfide), these may only be produced in the quantities necessary for the experiment. in the case of volatile substances, the experiment must be conducted under a hood where the gas can be drawn off. Residue must be appropriately disposed of after the experiment and may on no account be stored. If the facilities for disposal are not available, the experiment may not be conducted.

Less toxic substances and preparations may be obtained without a permit. Less toxic substances are also those which contain a highly toxic or toxic substance at a level of concentration below that determined by law as the maximum for classification as noxious. Chlorine water, bromine water and hydrogen sulfide solution in a concentration of up to 1% may therefore be used in instruction.

5. Corrosives and irritants (C, X i)

Caustic or corrosive substances (R 34, R 35), designation C, are those which can destroy living materials by their action upon it. Substances are classed as irritants (R 36 R 38), designation Xi, if they cause inflammation without being corrosive on direct, prolonged or repeated contact with the skin or mucous membranes. The relevant safety recommendations (S 22 S 28) should be observed.

6. Carcinogenic, genotype or embryo damaging, chronically harmful substances

Substances may not be used for instruction if they have a proven carcinogenic effect (R 45), if they cause hereditary damage (R 46) or embryo damage (R 47), or if they are chronically damaging (R 48), particularly those substances classed as unmistakably carcinogenic. Such substances must be removed from all school stocks. Storage is not permitted under any circumstances.

Further, substances for which there is a well founded suspicion of carcinogenic potential (R 40) may only be used if corresponding safety precautions are taken and only in such cases where they cannot be replaced by less dangerous chemicals.

B 2: R RATINGS AND S PROVISIONS

Nature of special risks (R)

R 1 Explosive when dry.
R 2 Risk of explosion by shock, friction, fire or other sources of ignition.
R 3 Extreme risk of explosion by shock, friction, fire or other sources of ignition.
R 4 Forms very sensitive explosive metallic compounds.
R 5 Heating may cause an explosion.
R 6 Explosive with or without contact with air.
R 7 May cause fire.
R 8 Contact with combustible material may cause fire.
R 9 Explosive when mixed with combustible material.
R 10 Flammable.
R 11 Highly flammable.
R 12 Extremely flammable.
R 13 Extremely flammable liquefied gas.
R 14 Reacts violently with water.
R 15 Contact with water liberates highly flammable gases.
R 16 Explosive when mixed with oxidizing substances.
R 17 Spontaneously flammable in air.
R 18 In use, may form flammable/explosive vapor air mixture.
R 19 May form explosive peroxides.
R 20 Harmful by inhalation.
R 21 Harmful in contact with skin.
R 22 Harmful if swallowed.
R 23 Toxic by inhalation.
R 24 Toxic in contact with skin.
R 25 Toxic if swallowed.
R 26 Very toxic by inhalation.
R 27 Very toxic in contact with skin.
R 28 Very toxic if swallowed.
R 29 Contact with water liberates toxic gas.
R 30 Can become highly flammable in use.
R 31 Contact with acids liberates toxic gas.
R 32 Contact with acids liberates very toxic gas.
R 33 Danger of cumulative effects.
R 34 Causes burns.
R 35 Causes severe burns.

R 36 Irritating to eyes.
R 37 Irritating to respiratory system.
R 38 Irritating to skin.
R 39 Danger of very serious irreversible effects.
R 40 Possible risks of irreversible effects.
R 41 Danger of serious eye damage.
R 42 May cause sensitization by inhalation.
R 43 May cause sensitization by skin contact.
R 44 Risk of explosion if heated by occlusion.
R 45 May cause cancer.
R 46 May cause hereditary damage.
R 47 May cause embryo damage.
R 48 Danger of chronic damage.

Safety advice (S)

S 1 Keep locked up.
S 2 Keep out of reach of children.
S 3 Keep in a cool place.
S 4 Keep away from living quarters.
S 5 Keep contents under .... (appropriate liquid to be specified by the manufacturer).
S 6 Keep under .... (inert gas to be specified by the manufacturer).
S 7 Keep container tightly closed.
S 8 Keep container dry.
S 9 Keep container in a well ventilated place.
S 10 Keep contents wet.
S 11 Avoid contact with air.
S 12 Do not keep the container sealed.
S 13 Keep away from food, drink and animal feeding stuffs.
S 14 Keep away from .... (incompatible materials to be indicated by the manufacturer).
S 15 Keep away from heat.
S 16 Keep away from sources of ignition No smoking.
S 17 Keep away from combustible materials.
S 18 Handle and open container with care.
S 20 When using do not eat or drink.
S 21 When using do not smoke.
S 22 Do not inhale dust.
S 23 Do not inhale gas/fumes/vapor/spray.
S 24 Avoid contact with skin.
S 25 Avoid contact with eyes.
S 26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S 27 Take off immediately all contaminated clothing.
S 28 After contact with skin, wash immediately with plenty of .... (to be specified by the manufacturer).
S 29 Do not empty into drains.
S 30 Never add water to this product.
S 31 Keep away from explosive materials.
S 33 Take precautionary measures against static discharges.
S 34 Avoid shock and friction.
S 35 This material and its container must be disposed of in a safe way.

S 36 Wear suitable protective clothing.
S 37 Wear suitable gloves.
S 38 In case of insufficient ventilation, wear suitable respiratory equipment.
S 39 Wear eye/face protection.
S 40 To clean the floor and all objects contaminated by this material, use .... (to be specified by the manufacturer).
S 41 In case of fire and/or explosion do not breathe fumes.
S 42 During fumigation/spraying wear suitable respiratory equipment.
S 43 In case of fire, use .... (indicate in space the precise type of fire fighting equipment. If water increases the risk, add Never use water).
S 44 If you feel unwell, seek medical advice (show the label where possible).
S 45 In case of accident or if you feel unwell, seek medical advice (show the label a where

B 3: EXPLANATION OF DANGER SYMBOLS

toxic (T) substances
and
very toxic (T+) substances

flammable (F) substances
and
extremely flammable (F+) substances

irritating (Xi) substances
and
harmful (Xn) substances

explosive (E) substances

oxidizing (O) substances

corrosive (C) substances

environmentally dangerous (N) substances

PRACTICAL PROBLEMS

Problem 28. Determination of copper and zinc by complexometric titration

Alloys can be found in many objects we come across in our daily life. Due to their particular characteristics (i.e., conductivity, mechanical or corrosion resistance), alloys are successfully applied in many advanced fields such as aeronautics, construction, electronics devices, and jewelry. That is why developing reliable methods of alloys analysis is of extreme importance.

Brass is an alloy of copper and zinc which is familiar to most students. In this experiment, a brass alloy containing Cu^{2+} and Zn^{2+} ions will be analyzed by complexometric titration with Na_2H_2EDTA. Since the stability constants of the complexes of these metals with EDTA are close, masking of the Cu^{2+} ions by a complexing agent (thiosulfate) is used. In the first titration, copper and zinc are titrated together with Na_2H_2EDTA. In the second titration, sodium thiosulfate is added to bind the Cu^{2+} ions, thus allowing titration of solely zinc ions with Na_2H_2EDTA.

Chemicals and reagents:

- Brass sample, ~250 mg per student, *or*
- Test solution (a standard solution containing about 1.5 g L^{-1} Cu^{2+} and 1 g L^{-1} Zn^{2+} ions simulating a digested sample of brass)
- Nitric acid, HNO_3, concentrated (~70% w/v)
- Na_2H_2EDTA standard solution, 0.0500 mol L^{-1}
- Acetate buffer solution, pH 5.5–6.0, 0.1 mol L^{-1} in acetate
- Sodium thiosulfate solution, $Na_2S_2O_3$, ~10% (w/v)
- Metallochromic indicator 4-(2-pyridylazo)resorcinol (PAR)[1], 0.1% aqueous solution (w/v)

Substance	State	R-Ratings	S-Provisions
$Cu(NO_3)_2$	aqueous solution	36 38	26
$Zn(NO_3)_2$	aqueous solution		24 25
HNO_3	aqueous solution	8 35	23 26 36 45
Na_2H_2EDTA	aqueous solution	36 37 38	26 37 39
$Na_2S_2O_3$	aqueous solution		24 25

Apparatus and glassware:

- Analytical balance (± 0.0001 g)
- Beaker, 10 mL
- Hotplate
- Volumetric flask, 100 mL

[1] 0.1% solution of Xylenol orange indicator may be used instead of PAR

- Burette, 25 or 50 mL
- Volumetric pipettes, 2, 5 and 10 mL
- Erlenmeyer flask, 100 mL (3 ea.)
- Graduated cylinders, 10 and 25 mL

A. Brass digestion

a) Take a precise weight of the brass sample (~250 mg) and place it in a beaker.

Note. *If no certified brass samples are available, you can use a test solution simulating the digested alloy.*

b) Carefully add 5 mL of concentrated nitric acid (the experiment should be done under a fume hood, as NO_2 gas evolves).

c) Heat the beaker slightly on a hotplate to provide for an effective dissolution.

d) When the digestion of the sample is complete, evaporate the solution to near dryness to remove the most part of the acid (avoid evaporating to dry salts, as hydrolysis may occur. If still so, add a minimal amount of HCl to dissolve the residue). Allow the beaker cooling down to room temperature.

e) Dissolve the contents of the beaker in distilled water, transfer it to a 100.00 mL volumetric flask and make it up to the mark.

B. Determination of the total amount of Cu^{2+} and Zn^{2+}

f) Transfer 10.00 mL of the test solution into a 100 mL Erlenmeyer flask, add 20 mL of water, 5 mL of acetate buffer solution and 3 drops of PAR solution, mix thoroughly.

g) Titrate the content of the flask with 0.0500 mol L^{-1} standard Na_2H_2EDTA solution until the color of PAR indicator changes from bluish-violet to blue or greenish-yellow (for Xylenol orange indicator, the color changes from red to green). Repeat the titration as necessary.

C. Determination of Zn^{2+}

h) Transfer 10.00 mL of the test solution into a 100 mL Erlenmeyer flask, add 10 mL of water, 5 mL of acetate buffer solution, 2 mL of $Na_2S_2O_3$ solution and 3 drops of PAR solution, mix thoroughly.

i) Titrate the content of the flask with 0.0500 mol L^{-1} standard Na_2H_2EDTA solution until the color changes from red to yellow (for Xylenol orange, the colors are the same).

D. Calculation of Cu^{2+} concentration

j) The volume of Na_2H_2EDTA which is necessary for Cu^{2+} titration is calculated as the difference of the titrant volumes in titrations **B** and **C**.

Questions and Data Analysis

1. Give balanced chemical equations for the reactions that take place when:
   - brass dissolves in nitric acid;
   - copper and zinc ions are titrated by Na_2H_2EDTA;
2. Explain how $Na_2S_2O_3$ masks the Cu^{2+} ion, giving the appropriate chemical equation.
3. Why should the pH value of the titrated solution be kept within 5–6?
4. Calculate the molar fraction of H_2EDTA^{2-} at pH 6. EDTA is a weak acid with the following acidity constants: $K_1 = 1.0 \cdot 10^{-2}$, $K_2 = 2.1 \cdot 10^{-3}$, $K_3 = 6.9 \cdot 10^{-7}$, $K_4 = 5.5 \cdot 10^{-11}$.
5. Derive the formulae for calculation of Cu^{2+} and Zn^{2+} concentrations in the test solution. Calculate the mass ratio of Cu and Zn in the alloy.

Problem 29. Conductometric determination of ammonium nitrate and nitric acid

Conductometric titration is a type of titration in which the electrical conductivity of the reaction mixture is continuously monitored as one reactant is added. The equivalence point in such titration is determined by the change in electrical conductivity of the solution. Marked jumps of conductance are primarily associated with changes of concentrations of the two most highly conducting species, hydrogen and hydroxyl ions. The method can be used for titrating colored solutions or suspensions, the latter being impossible with color indicators. Electrical conductivity measurement is used as a tool to locate the endpoint.

Industrial production of ammonium nitrate involves the acid-base reaction of ammonia with nitric acid. Conductometric titration can be used to control the residual concentration of nitric acid in the solution after the reaction with ammonia.

In this work you will perform a conductometric titration of a mixture of nitric acid and ammonium nitrate.

Table of Chemicals:

Compound	**State**	**R-Ratings**	**S-Provisions**
HNO_3	Solution in water, ~1 mol·L^{-1}	24 25 34	23 26 36 37 39 45
NH_3(aq)	Solution in water, ~1 mol·L^{-1}	10 23 34 37 41 50	23 24 25 26 36 37 39 45
NaOH(aq)	Solution in water, ~1 mol·L^{-1}	35	26 37 39 45
NaCl	Solid, 0.6 g	-	24/25

Equipment and Glassware:

- Conductivity meter
- Analytical balance (± 0.0001 g)
- Burette
- Volumetric pipettes, 10, 15 and 25 mL
- Pipette bulb or pump
- Magnetic stirrer
- Stirring bar
- Volumetric flasks, 100 mL (5 ea.)
- Glass beaker, 100 mL

Directions:

a) Place ammonia and nitric acid solutions into three 100-mL volumetric flasks marked **A**, **B**, and **C** in quantities indicated in the hereunder table. Fill the flasks with deionized water up to the mark and mix thoroughly.

Solution	Volume of 1 mol·L^{-1} HNO_3, mL	Volume of 1 mol·L^{-1} NH_3, mL
A	10	15
B	10	10
C	20	10

b) Transfer 25.0 mL of solution **A** into a glass beaker using a 25 mL transfer pipette.

c) Titrate the sample solution with a standardized NaOH (~1 M, known exactly) by adding 0.2 mL portions of the titrant. After adding each titrant portion, stir the solution. Record the value of the electric conductivity when it becomes constant.

d) Titrate the sample solution until the conductivity starts to rise (add a few more titrant portions to be able to draw a straight line).

e) Repeat steps (b – d) for solutions **B** and **C**.

f) Transfer 20 mL of HNO_3 and 10 mL of NH_3 solutions into each of volumetric flasks **D** and **E**. Fill the flasks up to the mark and mix thoroughly. For flasks filling, use distilled (instead of deionized) water for **D** and deionized water containing 0.6 g of NaCl for **E**.

g) Repeat steps (b – d) for solutions **D** and **E**.

Questions and Data Analysis

1. Give balanced chemical equations for the reactions taking place when the titrant is added.

2. Draw the titration curve in the coordinates "*electrical conductivity – volume of titrant*" for all the solutions studied (**A – E**). How many breaks of titration curves should be observed? Explain the resulting dependences. Which curves are practically the same and why?

3. Draw straight lines through the linear portions of the titration curves. Find the inflection points as the abscissa values corresponding to the intersections of the lines.

4. Calculate the concentrations of nitric acid and ammonium salt using these inflection points for each case. Compare the results with those calculated from the known amounts of HNO_3 and NH_3.

5. Using the obtained results, predict the curve shape for the titration of a mixture of sodium hydroxide and free ammonia with HCl.

Problem 30. Analysis of fire retardants by potentiometric titration

The purpose of the experiment is to determine the composition of a mixture simulating a fire retardant containing $(NH_4)_2HPO_4$ and NH_4Cl. First, the sample is dissolved in HCl and titrated with NaOH to determine the amount of phosphoric acid, the best precision being achieved if potentiometric titration (pH values recorded with a pH meter) is used. Generally, titration of a mixture of hydrochloric and phosphoric acids with an alkali results in two end points (inflexions in the titration curve). The first end point indicates the total amount of hydrochloric and phosphoric acids, while the second one corresponds to the completion of the second stage neutralization of phosphoric acid. In this experiment, the second end point cannot be observed due to the formation of ammonium buffer.

To determine the concentration of the ammonium salt, the formaldehyde method is used. The reaction between formaldehyde and ammonium produces the hexamethylene tetrammonium cation $(CH_2)_6(NH^+)_4$, which is more acidic than the NH_4^+cation. Another potentiometric titration

is necessary to find the total amount of $(CH_2)_6(NH^+)_4$, and thus calculate the total amount of diammonium phosphate and ammonium chloride in the sample.

The acidity constants of phosphoric acid: $K_{a1} = 7.1\times10^{-3}$, $K_{a2} = 6.2\times10^{-8}$, $K_{a3} = 5.0\times10^{-13}$.

Chemicals and Reagents

- Mixture of $(NH_4)_2HPO_4$ and NH_4Cl, about 1:1 by weight
- Sodium hydroxide, 0.1 M NaOH (aq)
- Hydrochloric acid, 0.1 M HCl (aq)
- Formaldehyde, 20 % CH_2O (aq)

Table of Chemicals

Compound	State	R-Ratings	S-Provisions
$(NH_4)_2HPO_4$	Solid	36/37/38	26
NH_4Cl	Solid	22 36	22
HCl	Liquid	23 25 34 38	26 36 37 39 45
NaOH	Liquid	35	26 37 39 45
CH_2O	Liquid	23/24/25 34 40 43	1/2 26 36/37/39 45 51

Equipment and Glassware

- Analytical balance (± 0.0001 g)
- Volumetric pipette, 10 mL
- Pipette pump
- Burette, 25 mL
- Beaker, 100 mL
- Volumetric flask, 100 mL
- Magnetic stirrer
- Stirring bar
- pH meter

A. Determination of phosphate amount as phosphoric acid

a) Weigh about 0.6 g of the test mixture and place it in a 100 mL volumetric flask. Fill with water up to the mark.

b) Transfer 10 mL of the prepared solution into a 100 mL beaker using a 10 mL volumetric pipette. Add 10 mL of 0.1 M hydrochloric acid (concentration known exactly) using a 10 mL volumetric pipette, and dilute it with 20 mL of distilled water. Place the beaker onto a magnetic stirrer and put in the stirring bar.

c) Titrate the sample with 0.1 M sodium hydroxide adding it by 0.5 mL portions until the pH starts increasing. Continue adding the titrant in drop portions. When the change of pH with each added portion significantly decreases, continue titration with larger portions of sodium hydroxide. Record the volume of sodium hydroxide added and each pH value measured.

d) Repeat the titration with new aliquots of the sample solution as needed to obtain consistent results.

B. Determination of the total amount of ammonium salts

e) Prepare a 20% aqueous solution of formaldehyde free of formic acid. Neutralize the solution with sodium hydroxide, if needed. Use titration in the presence of phenolphthalein to determine the necessary amount of NaOH for the neutralization.

f) Transfer 10 mL of the sample solution into a 100 mL beaker using a 10 mL volumetric pipette. Add 5 mL of the formaldehyde solution and wait for 2 min.

g) Place the beaker onto the magnetic stirrer and put in the stirring bar. Titrate the sample with 0.1 M sodium hydroxide with constant stirring as described in part **A**.

h) Repeat the titration with new aliquots of the sample solution as needed to obtain consistent results.

Questions and Data Analysis

1. How many end points are expected during the titration of a mixture of H_3PO_4 and HCl?
2. Can color indicators be used in the determination of concentrations of hydrochloric and phosphoric acids in their mixture?
3. Write down the equations of all the reactions occurred.
4. Plot the graphs of pH, $\Delta pH/\Delta V$, and $\Delta^2 pH/\Delta V^2$ *vs*. volume of the titrant added. Find the end points from the curves analysis. Why is there only one end point in the titration curve of hydrochloric and phosphoric acids in the presence of ammonium ion?
5. Calculate the content (in weight %) of (a) diammonium phosphate and (b) ammonium chloride in the test sample.

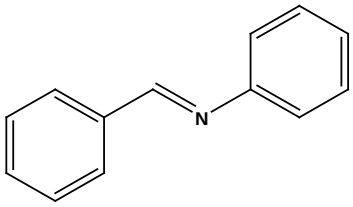
Problem 31. Formation of double carbon-nitrogen bond

Imines (nitrogen analogues of carbonyl compounds) are formed when any primary amine reacts with aldehyde or ketone under appropriate conditions. Mechanically, the amine first attacks the aldehyde with formation of an intermediate. Its subsequent dehydration gives the imine.

Imine formation is like a biological reaction: it is fastest near neutrality. Many biological processes involve imine formation. Three outstanding examples are: synthesis of amino acids from oxoacids, transamination of α-amino acids and mechanism of vision. The former two

processes include formation of an imino intermediate between an amino acid and vitamin B_6 derivative (pyridoxal). The transformation of light energy into electric signal in our eyes includes the *cis*-*trans*-photoisomerization of a polyene retinal (an aldehyde), which is covalently linked to the protein (an amine) by the imine bond. Imines are also very important in organic synthesis as intermediates in the so-called "reductive amination" reaction allowing direct transformation of carbonyl compounds into amines.

In this task you will prepare aniline derivative of benzaldehyde (I).



Chemicals and Reagents

- Aniline
- Benzaldehyde
- 96% aqueous ethanol

Table of Chemicals

Compound	State	R-Ratings	S-Provisions
C_6H_7N, Aniline	Liquid	23/24/25 40 41 43 48/23/24/25 50 68	26 27 36/37/39 45 46 61
C_7H_6O,Benzaldehyde	Liquid	22	2 24
C_2H_6O, Ethanol, 96% aqueous solution	Liquid	11	2 7 16

Equipment and Glassware

- Magnetic stirrer with heating
- Magnetic bar
- Glass beaker, 25 mL
- Round-bottom two necked flask, 50 mL
- Reflux condenser
- Laboratory stand with metal rings and clamps
- Adding funnel
- Separating funnel
- Filter flask
- Porous Shott's glass filter
- Water- or vacuum pump
- Analytical balance (± 0.001 g)
- Capillary for melting point determination (2-3 ea.)
- Glass tube for capillary filling
- Melting point apparatus
- Glass rod

- Ice bath

Procedure

***N*-[(*E*)-Phenylmethylene]aniline**

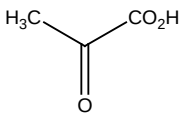
0.42 g of freshly distilled benzaldehyde is placed in a round bottom two necked flask equipped with a reflux condenser and an addition funnel. The reaction vessel is mounted on the magnetic stirrer with a heating mantle. 0.37 g of freshly distilled aniline is poured in the funnel. The aniline is added dropwise to the flask with intensive stirring. Almost immediately the yellow precipitate starts to form and the reaction mixture warms up. After the addition of aniline is finished, the reaction mixture is stirred for 15 minutes. To the end of this process prepare a 25 mL glass with 3 mL of 96% ethanol. Transfer the reaction mixture from the flask to the glass, wash the flask with 1 mL of ethanol and add this to the glass. Then place the glass in an ice-bath for 10 minutes. Knead the content of the glass and transfer it on the glass Shott's filter. Turn on the water-pump, connect it to the filtration flask and filter the precipitate off. To provide for effective drying, keep the precipitate pressing with the glass rod from time to time until the mother liquor stops to drop down. Keep drying the product under vacuum for at least 10 min. Weigh the product and calculate the yield. Pick out a few crystals of the product for further determination of its melting point.

Determination of melting point

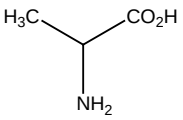
Use a glass capillary sealed from one side. Place the non-sealed end of the capillary into a product crystals, then turn it sealed end down and throw several times down through a glass tube. Check that the sealed side of the capillary is filled with the product. Apply the ready capillary to a melting point apparatus and record the melting point of the product.

Questions

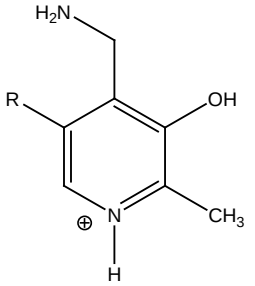
1. Draw the mechanism of imine formation. How can you name the intermediate? What are the rate-limiting steps at low and high pH conditions?
2. What is similar and different in mechanisms of imine and acetal formation?
3. Draw the mechanism of vitamin B_6 derivative catalyzed transformation of pyruvic acid into alanine.



pyruvic acid



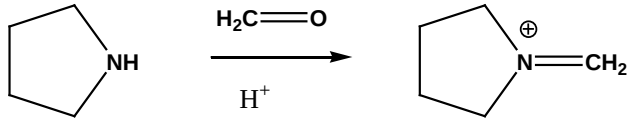
alanine

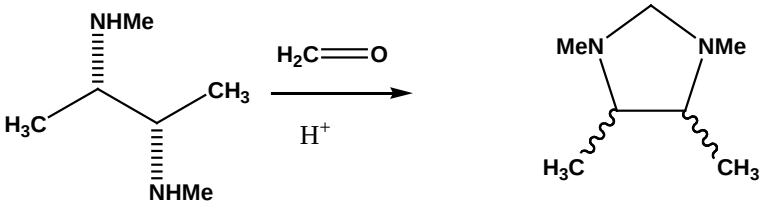


pyridoxamine phosphate, $R = CH_2OPO_3^{\ominus}H$

4. Draw the mechanism of reductive amination of cycohexanone into N,N-dimethyl cyclohexylamine using sodium cyanoborohydride and dimethylamine.

5. Suggest mechanisms for the two hereunder reactions. Draw the correct stereochemistry for the product of the second reaction.

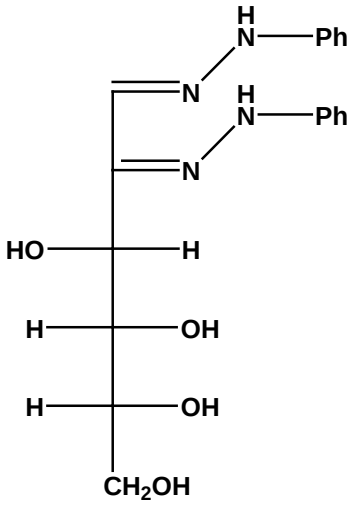




Problem 32. Osazone of glucose

Carbohydrates are in the very heart of biomolecular chemistry. Analysis of carbohydrates and products of their transformations is often hardly possible due to their appearance as oils or syrups with no characteristic melting point. The sophisticated stereochemistry of carbohydrates does not make their investigation easier. In the 1880th the German chemist Emil Fischer found that heating of some monosaccharides with an excess of phenylhydrazine results in formation of crystalline products, which he named “osazones”. Different phenylosazones existed as distinctive crystals, and formed at different rates from various parent sugars. The crystallinity of these products helped in their analysis, whereas the loss of chirality at the 2nd carbon atom was of

great importance in establishing stereochemical details of many monosaccharides. In this task you will prepare phenylhydrazine derivative of carbohydrate *D*-glucose (I).



Chemicals and Reagents

- *D*-Glucose
- Phenylhydrazine
- Water
- Acetic acid solution, 50%
- Ethanol, 96%

Table of Chemicals

Compound	State	R-Ratings	S-Provisions
$C_6H_{12}O_6$, *D*-Glucose	Solid	-	-
$C_6H_8N_2$, Phenylhydrazine	Liquid	23/24/25 43 45 48/23/24/25 68	45 53
$C_2H_4O_2$, Acetic acid, 50% solution	Aqueous solution	10 35	23 26 45
C_2H_6O, Ethanol, 96% solution	Aqueous solution	11	2 7 16

Equipment and Glassware

- Magnetic stirrer with heating
- Magnetic bar
- Water bath
- Round-bottom flask, 50 mL
- Reflux condenser
- Laboratory stand with metal rings and clamps
- Filter flask
- Porous Shott's glass filter
- Water- or vacuum pump
- Analytical balance (± 0.001 g)
- Pipette pump
- Capillary for melting point determination (2-3 ea.)

- Glass tube for capillary filling
- Melting point apparatus
- Glass rod

Procedure

***D*-glucose osazone**

To a round bottom flask equipped with a reflux condenser and a water bath add 200 mg of glucose, 4 mL of water, 400 mg of freshly distilled phenylhydrazine (caution – poisonous!) and 0.4 mL of 50% acetic acid. Using the magnetic stirrer with a heating mantle, heat the reaction mixture until the water in the bath starts boiling. In 5 min, the yellow precipitate of osazone will start forming. Continue heating for 1 h, then carefully remove the bath, remove the condenser and let the reaction mixture slowly cool down to the room temperature.

Knead the content of the flask and transfer it on the glass Shotts' filter. Turn on the water-pump, connect it to the filtration flask and filter the precipitate off. After the mother liquor stops dropping down, disconnect the flask and take the glass filter off. Wash the reaction flask with mother liquor, place the glass filter back, pour the content of the reaction flask onto the filter, and connect to vacuum. After the mother liquor stops dropping down, disconnect the flask. Add 3 mL of ethanol to the precipitate, knead it with a glass bar, and connect to vacuum again. Repeat the rinsing procedure with ethanol once more. To provide for effective drying, keep the precipitate pressing with the glass rod from time to time. Keep drying the product under vacuum for at least 10 min. Weigh the product and calculate the yield. Pick out a few crystals of the product for further determination of its melting point.

Determination of melting point

Determine the melting point of the product according to the directions in Problem 31.

Questions

1. Put the stoichiometry coefficients for the reaction between *D*-glucose and phenylhydrazine. What are the other products of this reaction?

2. Which starting substance would you use to calculate the yield of your product?

3. What is the product of the glucose reaction with equimolar amount of phenylhydrazine under mild conditions?

4. Draw the osazones of *D*-glucose, *D*-mannose and *D*-fructose. What can you say about the similarity in stereochemistry of the starting sugars?

5. Do the pairs of osazones of the hereunder sugars represent the same or different molecules?

a) *D*-glucose and *L*-glucose

b) *D*-allose and *D*-talose

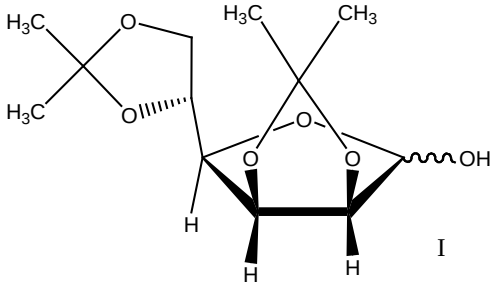
c) *D*-galactose and *D*-talose

d) *D*-ribose and *D*-allose

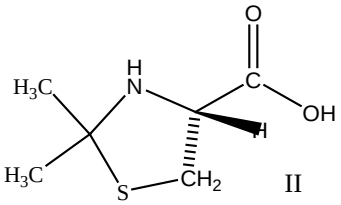
Problem 33. Acetone as a protecting agent

Protecting groups play significant role in modern organic synthesis, since they allow hiding the reactive X-H groups (X = O, N, S) from interaction with, mainly, nucleophilic and oxidizing reagents. At the same time, protecting groups are further easily removed by applying specific reagents under mild conditions. Acetone, commonly known as an organic solvent, is also widely used in organic synthesis as a protecting agent. Acetone reveals a broad spectrum of the reaction ability towards hydroxyl, amino and thiol groups forming either hemiketals or ketals (and their N- and S-analogues) depending on the number and location on nucleophilic X-H groups. In the form of its (hetero)ketal, the acetone residue can be considered in the protected molecule as part of a five-membered saturated 1,3-diheterocycle.

In this task you will prepare acetone derivatives of carbohydrate *D*-mannose (I) and α-amino acid *L*-cysteine (II).



I



II

Chemicals and Reagents

- *D*-Mannose
- Iodine, crystalline
- Anhydrous acetone
- $Na_2S_2O_3$ solution, dilute

- Chloroform
- Na_2SO_4, calcined
- *L*-Cisteine hydrochloride
- Ninhydrine reagent (0.3 % sol-n of ninhydrine in n-butanol cont. 3% of sodium acetate)

Table of Chemicals

Compound	State	R-Ratings	S-Provisions
$C_6H_{12}O_6$, *D*-Mannose	Solid	-	28
I_2	Solid	20/21 50	23 25 61
$CH_3C(O)CH_3$	Liquid	11 36 66 67	2 9 16 26
$Na_2S_2O_3$	Aqueous solution	-	24/25
$CHCl_3$	Liquid	22 38 40 48/20/22	2 36/37
Na_2SO_4	Solid	-	-
$C_3H_8NO_2SCl$, *L*-Cisteine hydrochloride	Solid	22 36/37/38	25 26 36/37/39
$C_9H_6O_4$, Ninhydrine	Solution	22 36/37/38	26 28A 37/39
C_4H_9OH, n-butanol	Liquid	10 22 37/38 41 67	2 7/9 13 26 37/39 46
CH_3COONa	Solution	-	-

Equipment and Glassware

- Magnetic stirrer with heating
- Magnetic bar
- Glass beaker, 50 or 100 mL (2 ea.)
- Round-bottom flask, 50 mL
- Reflux condenser
- Laboratory stand with metal rings and clamps
- Thermometer
- Adding funnel
- Separating funnel
- Filter flask
- Porous Shott's glass filter (2 ea.)
- Rotary evaporator
- Water- or vacuum pump
- Analytical balance (± 0.001 g)
- Pipette pump
- Capillary for melting point determination (2-3 ea.)
- Glass tube for capillary filling
- Melting point apparatus
- Filter paper
- Glass rod
- Ice bath

Procedure

A. *D*-Mannose protection with acetone

Fix a beaker on a magnetic stirrer with a metal ring attached to a stand. Place 200 mg of mannose, 60 mg of crystalline iodine and 12 mL of anhydrous acetone in the beaker. Attach to stand a thermometer with its bulb in the reaction mixture. Heat the reaction mixture for *ca.* 30 min at 35°C with stirring. After all the mannose is dissolved, turn the heater off and cool the mixture down to the room temperature. Then fix an adding funnel above the beaker using a metal ring attached to the stand (take care the stopcock is closed!). Pour the dilute $Na_2S_2O_3$ solution into the funnel and add it dropwise to the brown reaction mixture until the color disappearance. Add 10 mL of water and transfer the reaction mixture from the beaker into a separating funnel (take care the stopcock is closed!) fixed on the stand using a metal ring. Add 10 mL of chloroform and close the funnel by placing the stopper at its top. Take the funnel in your hands so that its narrow end is directed upwards and away from yourself. Carefully turn the stopcock, release the air and close the funnel back. Shake the funnel several times with agitation and release the air as described above. Repeat shaking and air release three times. Then hang the funnel back on the metal ring and wait until the aqueous and organic layers are clearly separated. Remove the stopper from the top of the funnel. Carefully open the stopcock and let the lower organic layer to flow into a beaker. Leave the upper aqueous layer in the funnel. Add another 10 mL of chloroform to the funnel and repeat the extraction procedure using the same beaker. Wash the combined organic layers with 10 mL of water using a clean separation funnel. Place calcined Na_2SO_4 into the beaker with combined organic layers. Fix the beaker on the magnetic stirrer, add the magnetic bar and stir the mixture for 15 min. Filter the drying agent off. Remove the solvent from the filtrate using a rotary evaporator[2]. Weigh the obtained white product and calculate the yield. Pick out a few crystals of the product for further determination of its melting point.

B. Modification of *L*-Cisteine with acetone

Fix a round-bottom flask on a stand. Place 100 mg of *L*-cisteine hydrochloride in 2 mL of anhydrous acetone in the flask. Attach the reflux condenser and heat the mixture to boiling. The starting amino acid hydrochloride readily dissolves, which is shortly followed by the product precipitation. Keep refluxing for about 30 min, then remove the condenser and cool down the reaction mixture using an ice bath. Knead the content of the flask and transfer it onto the glass Shott filter. Turn on the vacuum or water-pump, connect it to the filtration flask and filter the precipitate off. After the mother liquor stops dropping down, disconnect the flask and take the

[2]Can be done by a lab assistant. Students need not be trained in rotary evaporation.

glass filter off. Rinse the reaction flask with the mother liquor, place the glass filter back, pour the content of the reaction flask onto the filter, and connect to the vacuum line. After the mother liquor stops dropping down, disconnect the flask. Add 1 mL of anhydrous acetone to the precipitate, knead with a glass rod, and connect the flask to the vacuum line again. To provide for effective drying, keep the precipitate pressing with the glass rod from time to time. Keep drying the product under vacuum for at least 10 min. Pick out a few crystals of the product for further determination of its melting point.

Test reaction

Do the following test to check whether the reaction of cysteine protection with acetone is complete.

Ninhydrine reaction. Dissolve several milligrams of the product in aqueous acetone, and immediately apply a drop of the resulting solution to filter paper. Cover the spot with a drop of ninhydrine reagent. Gently heat up the filter paper. Perform the same test with the starting amino acid. Compare the results and explain the difference.

Determination of melting point

Determine the melting points of the products according to the directions in Problem 31.

Questions

1. Draw the mechanism of formation of 1,3-dioxolane ring from acetone and 1,2-diol. Which catalyst acid or base, will you apply? Why?

2. Draw the products of acetone reaction with *trans*- and *cis*-cyclohexane-1,2-diols. Which of the products is thermodynamically more favorable?

3. Based on the answer to Question 2, explain the nature and stereochemistry of the product of the *D*-mannose reaction with acetone paying attention to the mutual stereochemical relationships between vicinal hydroxyl groups in the starting sugar. Why the initial six-membered pyranose transforms into five-membered furanose? What is the way of such transformation in carbohydrate chemistry?

4. What conditions and reagents would you apply to remove acetone protecting groups from diacetonemannose?

5. Draw the mechanism of product formation in the reaction of cysteine with acetone. Explain the role of hydrochloric acid.

6. Draw the mechanism and products of the reaction between cysteine and ninhydrine. Show the product which is responsible for the color of the reaction mixture.

Problem 34. Determination of molecular mass parameters (characteristics) by viscometry

Fluid resistance to flow is referred to as viscosity. It is quantitatively characterized by the viscosity coefficient (fluids with high viscosity coefficients reveal enhanced resistance to flow). Experimentally, the viscosity coefficient can be determined by following the rate at which a liquid flows out from a thin capillary.

The viscosity of solutions of low-molecular weight compounds only slightly depends on their concentration. By contrast, solutions of polymers are characterized by a pronounced dependence of their viscosity on the polymer concentration, which allows determining the latter from viscometry data analysis.

For dilute polymer solutions, it was found that the reduced viscosity η_{red} and polymer concentration c (in g/mL) are related as follows:

$$\eta_{red} = \frac{t - t_0}{t_0 c}.$$

where t and t_0 are flow times of the solution and pure solvent, respectively.

The intrinsic viscosity $[\eta]$ can be further determined from extrapolation of the reduced viscosity to zero polymer concentration:

$$\eta_{red}(c) = [\eta] + kc\,.$$

The intrinsic viscosity is a function of the polymer and solvent nature. In general, it is related to the molar mass of the polymer according to the Mark-Kuhn-Houwink equation:

$$[\eta] = KM^{a}$$

Increasing of the solvent-polymer affinity results in more expanded polymer coils, which, in turn, provides for higher resistance to the solution flow. Thus, the index of power (a) is growing with increasing of the solvent affinity towards the polymer.

Usually a polymer sample is polymolecular (polydisperse), i.e. it contains macromolecules of different molecular weights. Accordingly, polymer samples are characterized by average molar masses (depends on the way of averaging). Thus, a viscosity-average molar mass M_v can be found from the Mark-Kuhn-Houwink equation using experimentally determined [η] and reference data for K and a.

Polydispersity (or heterogeneity) index of a polymer sample can be determined as the ratio of its viscosity-average molar masses found in solvents significantly differing in their affinity towards the polymer.

In this task you will find the polydispersity index of a polystyrene sample by capillary viscometry using toluene (K=0.017 ml/g, a=0.69) and methyl ethyl ketone (K=0.039 ml/g, a=0.57). All constants are given for 25 °C.

Chemicals and reagents:

- Polystyrene (number-average molar mass of about 100 000) solution in toluene, 10g/L, 25 mL
- Polystyrene (number-average molar mass of about 100 000) solution in methyl ethyl ketone, 10g/L, 25 mL
- Toluene, 50 mL
- Methyl ethyl ketone, 50 mL

Table of Chemicals

Compound	State	R-Rating	S-Provision
$(C_8H_8)_n$, Polystyrene	Solutions in toluene and methyl ethyl ketone	-	-
C_7H_8, Toluene	Liquid	11 38 48/20 63 65 67	2 29 36/37 46 62
C_4H_8O, Methyl ethyl ketone	Liquid	11 36 66 67	2 9 16

Apparatus and glassware:

- Ubbelohde or other capillary viscometer
- Graduated cylinder, 10 mL
- 10 glass vials, 20 mL

- Volumetric pipette, 5 mL
- Stopwatch

Procedure

a) For both polymer solutions, prepare a number of dilutions (in the concentrations range of 1 to 10 g/L).

b) Measure flow time for the solvent (toluene) using the Ubbelohde viscometer (repeat three times).

c) Measure flow times for all polystyrene solutions in toluene (repeat each three times)

d) Fill in the table below.

e) Repeat ii. b) – d) for polystyrene solutions in methyl ethyl ketone.

Concentration of the polymer c, g/L	Flow time t, s	$\eta_{rel} = \frac{t}{t_0}$	$\eta_{sp} = \frac{t - t_0}{t_0}$	$\frac{\eta_{sp}}{c}$, L/g
10				

Questions and data analysis

1. Calculate the relative, specific and reduced viscosities for each solution studied

2. Plot the reduced viscosity against polystyrene concentration for each solvent.

3. Approximate the dependences from i. 2 with appropriate straight lines.

4. Determine the intrinsic viscosity of the polystyrene solutions in toluene and methyl ethyl ketone as Y-intercept.

5. Using the Mark-Kuhn-Houwink equation, determine the corresponding values of viscosity-average molar masses of the polystyrene sample.

6. Evaluate the polydispersity index of the polystyrene sample.

Problem 35. Cooperative interactions in polymer solutions

Macromolecular interactions in solutions are behind many processes in living organisms. Organization of DNA into a double helix can serve as a well-known example. Formation of such intermolecular complexes is often driven by significant entropy gain. In laboratory this phenomena can be studied by using a simple model system, a mixture of poly(methacrylic acid) and poly(ethylene glycol).

Chemicals and reagents:

- (PMAA, molecular weight of 30000) aqueous solution, 2 g/L, 50 mL
- Poly(ethylene glycol) (PEG, molecular weights of 1000, 2000, 3000, 6000) aqueous solutions, 1 g/L, 10 ml of each solution
- Deionized water

Table of Chemicals

Compound	State	R-Ratings	S-Provisions
$(C_4H_6O_2)_n$, Poly(methacrylic acid)	Aqueous solution	-	-
$C_{2n}H_{4n+2}O_{n+1}$, Poly(ethylene glycol)	Aqueous solution	-	-

Apparatus and glassware:

- Ubbelohde viscometer or other capillary viscometer with thermostat
- Graduated cylinder, 10 mL
- 10 glass vials, 20 mL
- Volumetric pipette, 5 mL
- Stopwatch

Procedure

a) Prepare 1 g/L solution of PMAA in water by diluting the initial solution of PMMA.

b) Prepare mixtures of the initial solution of PMMA with the initial solutions of PEG of different molecular weights, each in volume ratio of 1:1 (4 mixtures in total).

c) Measure the flow time of water at 25°C using the Ubbelohde viscometer (repeat three times)

d) Measure the flow time of the prepared PMAA solution and of all mixtures at 25°C (repeat each three times).

e) Fill in the table below.

f) Repeat ii. c)-e) at 40°C.

Composition	Temperature, °C	Flow time, s	Specific viscosity of the solution
Water	25		
PMAA, 1 g/l	25		
PMAA+PEG-1000	25		
PMAA+PEG-2000	25		
PMAA+PEG-3000	25		
PMAA+PEG-6000	25		
water	40		
PMAA, 1 g/l	40		
PMAA+PEG-1000	40		
PMAA+PEG-2000	40		
PMAA+PEG-3000	40		
PMAA+PEG-6000	40		

Questions and data analysis

1. Calculate the specific viscosity (see the explanation in Problem 34) for each of the measured samples.

2. Plot specific viscosity against molecular weight of PEG for each temperature.

3. Explain the dependences of the viscosity on temperature and molecular weight of PEG.