

45th



International Chemistry Olympiad

PREPARATORY PROBLEMS

Edited by Anton Sirota

**27 theoretical problems
8 practical problems**

2013

THE PREPARATORY PROBLEMS FROM THE INTERNATIONAL CHEMISTRY OLYMPIADS, Series 1
The preparatory problems from the 45th IChO

Edited by Anton Sirota

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Preparatory problems

**45th International Chemistry Olympiad
(IChO-2013)**

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P r e f a c e

written by members of the IChO-2013 Science Committee
(a shortened version)

The members of the Science Committee really did their best to prepare interesting preparatory 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.

We pay great attention to safety. 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. During the Practical Examination at the 45th IChO very toxic chemicals will be used under no circumstances.

Members of the IChO-2013 Science Committee

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, the students are expected to be acquainted with basic synthetic techniques: vacuum filtration, drying of precipitates, determination of melting point and extraction with immiscible solvents.

THE FORTY-FIFTH INTERNATIONAL CHEMISTRY OLYMPIAD

15 – 24 JULY 2013, MOSCOW, RUSSIAN FEDERATION

PREPARATORY PROBLEMS

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PREPARATORY THEORETICAL PROBLEMS

THEORETICAL 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.

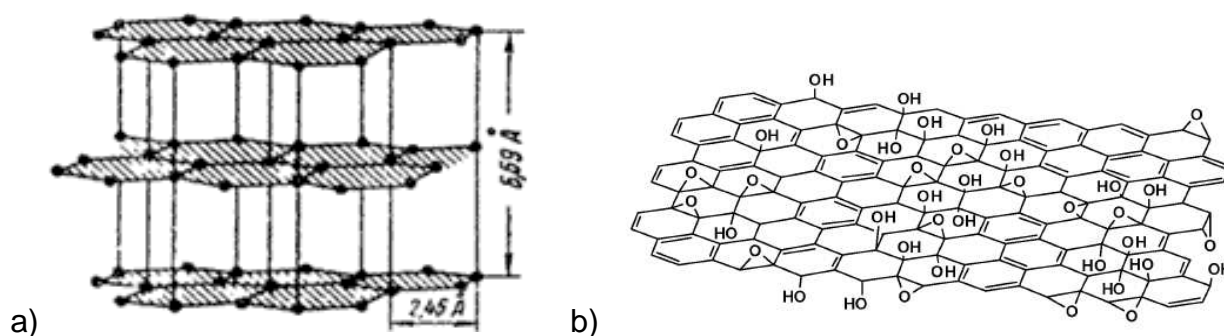


Figure 1. a) Crystal lattice of graphite. GO retains the layer structure of graphite, but the interlayer spacing is almost two times larger ($\sim 12 \text{ \AA}$ instead of 6.69 \AA in the figure) and part of the carbon atoms are oxidized. b) Single sheet in the GO crystal lattice. Several oxygen containing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

- 1.1 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?
- 1.2 The simplest model of the GO sheet (the Hoffman model) is presented in Fig. 2a. It was assumed that only one functional group, namely $(-\text{O}-)$ 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?

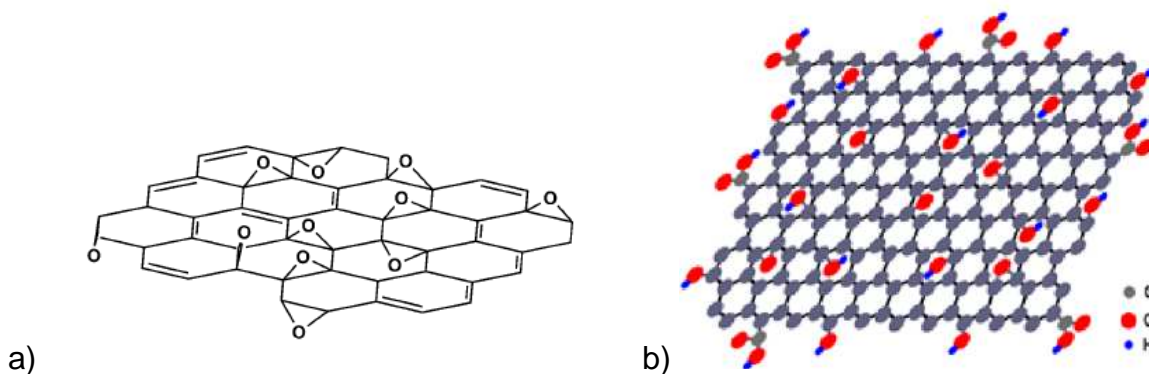


Figure 2. (a) Hoffman structural model of the GO sheet/ (b) Lerf-Klinowski model

- 1.3** 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.
- 1.4** 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 $\text{CH}_{0.22}\text{O}_{0.46}$. Estimate the amount of carbon atoms (in %) which were not oxidized. Give the upper and lower limits.
- 1.5** 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 $\text{CH}_{0.22}\text{O}_{0.46}$. 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).

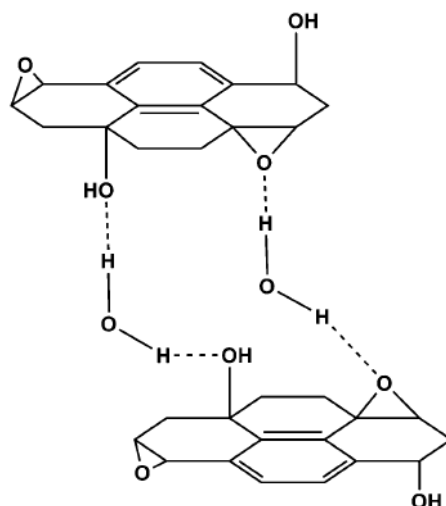


Figure 3. Proposed hydrogen bonding network formed between oxygen functionality on GO and water

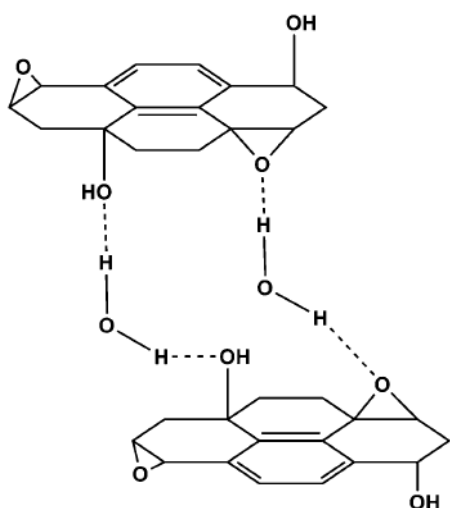


Figure 3.
Proposed hydrogen bonding network
formed between oxygen functionality on
GO and water

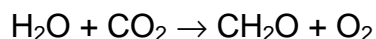
SOLUTION OF PREPARATORY PROBLEM 1

- 1.1** In GO the interplane spacing is larger. This facilitates exfoliation of GO. Graphite is hydrophobic, whereas GO is hydrophilic due to the formation of the functional groups. This makes GO soluble in water, which is very important for chemical exfoliation. The grave disadvantage of GO as a precursor of graphene is the necessity of reduction of single sheets after exfoliation. Graphene produced from GO is always defective.
- 1.2** 25 % of carbon atoms retain the sp^2 hybridization, which means that they are not bonded to oxygen atoms. 75% of carbon atoms form chemical bonds with oxygen. Each oxygen atom is bonded to the pair of carbon atoms. The net formula is $CO_{0.375}$. Maximum X in the Hoffman model is 0.5. The net formula is $CO_{0.5}$.
- 1.3** The four groups are the phenol ($OH\ sp^2$), hydroxyl ($OH\ sp^3$), and epoxide groups in the basal plane, and the carboxylic acid groups at the edges.
- 1.4** Each hydrogen atom corresponds to one oxidized carbon atom. 22 % of carbon atoms are bonded to the hydroxyl or phenol group, or are in the carboxylic acid group. Let all the hydrogen atoms be in the carboxylic acid groups. Then 44 % of oxygen atoms are in the carboxylic acid groups and 2% are in the epoxy groups. In this case $22\% + (2 \times 2)\% = 26\%$ of all the carbon atoms are oxidized. 74 % of the total amount of carbon atoms do not form chemical bonds with oxygen. This is the upper limit. Let all the hydrogen atoms be in the hydroxyl or phenol groups. This means that there are no carboxylic acid groups in the particular GO sample! Then 24 % of oxygen atoms are in the epoxy groups. In this case $22\% + 2 \times 24\% = 70\%$ of all the carbon atoms are bonded to oxygen. 30 % of carbon atoms are not oxidized. This is the lower limit.
- 1.5** Acid groups do not participate in the hydrogen bonding network (Fig. 3). It means that maximum degree of water absorption will be reached in case of the absence of such groups in GO. Then each pair of hydrogen atoms holds one molecule of H_2O (0.11), and each pair of epoxy groups also holds one molecule of H_2O $(0.46 - 0.22) / 2 = 0.12$. Altogether there are 0.23 molecules of water per one carbon atom. The chemical formula of GO hydrate is $CH_{0.22}O_{0.46} \cdot 0.23\ H_2O$.

THEORETICAL 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:



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 $1/6(\text{glucose})$. Using the information presented below, answer the following questions.

- 2.1 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.
- 2.2 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).
- 2.3 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.
- 2.4 How many photons will be absorbed and how much biomass (in kg) and oxygen (in m^3 at 25 °C 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)?
- 2.5 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 – $150 \text{ W}\cdot\text{m}^{-2}$;

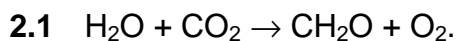
Moscow area – 1070 km^2 , percentage of green plants area – 18 %;

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 ₂ O(l)	CO ₂ (g)	O ₂ (g)	C ₆ H ₁₂ O ₆ (s)
Standard enthalpy of combustion, $\Delta_c H_{298}^{\circ}$, kJ·mol ⁻¹	–	–	–	–2805
Standard entropy, S_{298}° , J·K ⁻¹ ·mol ⁻¹	70.0	213.8	205.2	209.2

SOLUTION OF PREPARATORY PROBLEM 2



The process is reverse to combustion of 1/6(glucose), hence:

$$\Delta_r H_{298}^{\circ} = -\frac{1}{6} \Delta_c H_{298}^{\circ} (\text{C}_6\text{H}_{12}\text{O}_6) = 467.5 \text{ kJ mol}^{-1}$$

Standard entropy change in the reaction:

$$\Delta_r S_{298}^{\circ} = \frac{1}{6} S_{298}^{\circ} (\text{C}_6\text{H}_{12}\text{O}_6) + S_{298}^{\circ} (\text{O}_2) - S_{298}^{\circ} (\text{H}_2\text{O}) - S_{298}^{\circ} (\text{CO}_2) = -43.7 \text{ JK}^{-1} \text{ mol}^{-1}$$

Standard Gibbs energy change:

$$\Delta_r G_{298}^{\circ} = \Delta_r H_{298}^{\circ} - 298 \Delta_r S_{298}^{\circ} = 467.5 - 298 \times (-43.7 \cdot 10^{-3}) = 480.5 \text{ kJ mol}^{-1}$$

Energy of 1 mol of photons with wavelength of 680 nm:

$$E_m = \frac{hcN_A}{\lambda} = \frac{6.63 \cdot 10^{-34} \times 3.00 \cdot 10^8 \times 6.02 \cdot 10^{23}}{680 \cdot 10^{-9}} \times 10^{-3} = 176 \text{ kJ mol}^{-1}$$

The minimum number of photons necessary to supply more energy than $E = 480.5 \text{ kJ mol}^{-1}$ is **3**.

2.2 Energy of 10 mol of photons absorbed by green plants is $176 \cdot 10 = 1760$ kJ. Of this amount 480.5 kJ is converted to Gibbs energy. The efficiency of the solar energy conversion by green plants can be estimated as $480.5 / 1760 \cdot 100 \% = 27 \%$.

2.3 Total solar energy absorbed:

a) Moscow area: $E = 1070 \cdot 10^6 \text{ m}^2 \cdot 150 \text{ J s}^{-1} \cdot \text{m}^{-2} \cdot (10 \times 86400) \text{ s} = 1.4 \cdot 10^{17} \text{ J}$.

b) MSU campus: $E = 1.7 \cdot 10^6 \text{ m}^2 \cdot 150 \text{ J s}^{-1} \cdot \text{m}^{-2} \cdot (5 \times 3600) \text{ s} = 4.6 \cdot 10^{12} \text{ J}$.

Number of photons $N = (E / E_m) N_A$:

a) Moscow area: $N = 4.8 \cdot 10^{35}$.

b) MSU campus: $N = 1.6 \cdot 10^{31}$.

Solar energy utilized by green plants and converted to chemical energy:

a) Moscow area: $E_{\text{util}} = 1.4 \cdot 10^{17} \times (18\% / 100\%) \times (10\% / 100\%) \times (27\% / 100\%) = 6.8 \cdot 10^{14} \text{ J}$

b) MSU campus: $E_{\text{util}} = 4.6 \cdot 10^{12} \times (54\% / 100\%) \times (10\% / 100\%) \times (27\% / 100\%) = 6.7 \cdot 10^{10} \text{ J}$

Quantity of photosynthesis products $n(\text{CH}_2\text{O}) = E_{\text{util}} / \Delta_r G_{298}^\circ$

a) Moscow area: $n(\text{CH}_2\text{O}) = n(\text{O}_2) = 1.4 \cdot 10^9 \text{ mol}$

$$m(\text{CH}_2\text{O}) = n \cdot M = 1.4 \cdot 10^9 \text{ mol} \cdot 0.03 \text{ kg / mol} = 4.2 \cdot 10^7 \text{ kg}$$

$$V(\text{O}_2) = n \cdot V_m = 1.4 \cdot 10^9 \text{ mol} \cdot 0.0244 \text{ m}^3 / \text{mol} = 3.4 \cdot 10^7 \text{ m}^3$$

b) MSU campus: $n(\text{CH}_2\text{O}) = n(\text{O}_2) = 1.4 \cdot 10^5 \text{ mol}$

$$m(\text{CH}_2\text{O}) = n \cdot M = 1.4 \cdot 10^5 \text{ mol} \times 0.03 \text{ kg / mol} = 4200 \text{ kg}$$

$$V(\text{O}_2) = n \cdot V_m = 1.4 \cdot 10^5 \text{ mol} \times 0.0244 \text{ m}^3 / \text{mol} = 3400 \text{ m}^3$$

2.4 Percent of solar energy converted to chemical energy:

a) Moscow area: $(18\% / 100\%) \times (10\% / 100\%) \times (27\% / 100\%) = 0.005 = 0.5 \%$

b) MSU campus: $(54\% / 100\%) \cdot (10\% / 100\%) \cdot (27\% / 100\%) = 0.015 = 1.5 \%$

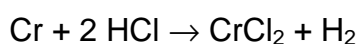
THEORETICAL PROBLEM 3

Ammine complexes of transition metals

- 3.1 The synthesis of chromium(III) ammine complexes usually starts from a freshly prepared *in situ* solution of a chromium(II) salt. How can one prepare such a solution using metallic chrome? Specify the conditions.
- 3.2 To the solution of a chromium(II) 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 % by mass of N. Determine the composition of the precipitate and give the reaction equation.
- 3.3 What oxidizer can be used instead of oxygen to obtain the same product? Justify the choice.
- 3.4 What product will be formed if the experiment described above is performed under inert atmosphere without oxygen? Give the equation.
- 3.5 Explain why the ammine complexes of chromium(III) cannot be prepared by the action of water ammonia on a solution of chromium(III) salt.
- 3.6 Arrange the hexammine complexes of iron(II), chromium(III) and ruthenium(II) in a row of increasing stability towards the acidic water solutions. Explain your choice.
- 3.7 In the case of $[\text{Ru}(\text{NH}_3)_6]^{2+}$ the hydrolysis rate increases upon the addition of an acid. Propose a mechanism and derive the rate law.

SOLUTION OF PREPARATORY PROBLEM 3

- 3.1 Chrome is dissolved in a diluted sulfuric or hydrochloric acid:

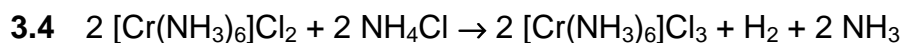


The experiment is conducted under inert atmosphere.

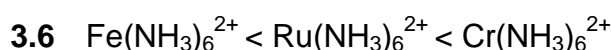
- 3.2 $4 [\text{Cr}(\text{NH}_3)_6]\text{Cl}_2 + 4 \text{NH}_4\text{Cl} + \text{O}_2 \rightarrow 4 [\text{Cr}(\text{NH}_3)_5\text{Cl}]\text{Cl}_2\downarrow + 4 \text{NH}_3 + 2 \text{H}_2\text{O}$

The formula of the precipitate is $\text{CrCl}_3\text{N}_5\text{H}_{15}$.

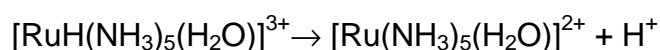
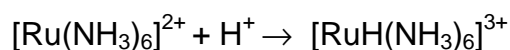
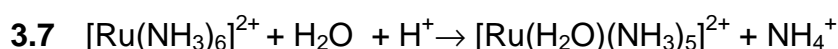
3.3 H_2O_2 . The compound $[\text{Cr}(\text{NH}_3)_5\text{Cl}]\text{Cl}_2$ is formed because the oxidation takes place via the η_2 -bridging peroxocomplex, followed by the hydrolysis when the leaving peroxy-group is replaced by the chloride-ion from the solution.



3.5 The chromium(III) complexes are inert, thus the substitution process occurs slowly. This is due to the d^3 configuration.



The coordinated ammonia has no vacant electron pair and therefore cannot interact with a proton. The iron(II) complex is labile, that is, ammonia ligands can be easily substituted by water molecules, which have a free electron pair even when linked to a metal atom. The ruthenium(II) complex is inert, but due to high atomic radius of ruthenium has a possibility to form an intermediate complex with an enhanced coordination number. The chromium(III) complex is inert and has no possibility to bind a proton. Therefore it is the most stable complex in the acidic media.



$$r = k [\text{H}^+] [\text{RuH}(\text{NH}_3)_6]^{2+}$$

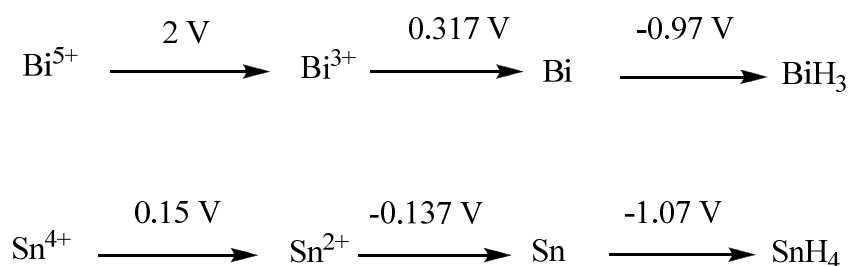
See J.D. Atwood, Inorganic and organometallic reaction mechanisms, 2nd edition, Wiley-VCH, pp.85-86 and P.C. Ford et al, Inorg. Chem., 1968, 7, 1976.

THEORETICAL 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 cm³) and concentrated sulfuric acid (4 cm³). 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 %.

- 4.1 Determine the composition of X.
- 4.2 What important instruction has been omitted in the description of the procedure?
- 4.3 Predict the structure of the cation in X taking into account that all the metal atoms in it are equivalent.
- 4.4 What particles are formed by addition of an acid or an alkali to the solution of X?
- 4.5 What happens when a solution of bismuth trichloride in HCl solution is added to the solution of tin chloride? The concentrations of all solutions are 1 mol dm⁻³. Calculate the equilibrium constant of the reaction. Extract the necessary data from the Latimer diagrams below.

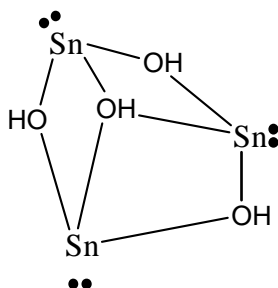


SOLUTION OF PREPARATORY PROBLEM 4

4.1 The common mineral of tin is cassiterite, SnO_2 . Thus, 1.05 g of **X** after decomposition give 0.8664 g of SnO_2 that contains 5.738 mmol of tin. Under decomposition 0.069 g (3.833 mmol) of water form. As the ratio $n(\text{Sn}) : n(\text{H}_2\text{O})$ is equal to 1.5 (or 3 : 2), the brutto formula of **X** contains 3 equivalents of SnO_2 , 4 of H and 2 of O (from 2 water molecules). In addition, it also contains nitrogen and probably more oxygen. Their mass is $1.05 - 0.8664 - 0.069 = 0.1146$ g and the average molar mass is $M = 0.1146 / (0.00383/2) = 60 \text{ g mol}^{-1}$, which corresponds to N_2O_2 . Thus, the formula of **X** is $\text{Sn}_3\text{O}_{10}\text{N}_2\text{H}_4$, or $\text{Sn}_3\text{O}_2(\text{NO}_3)_2(\text{H}_2\text{O})_2$.

4.2 All the operations should be performed in an inert atmosphere, because tin(II) hydroxide is oxidized in air.

If all the metal atoms in the cation are equivalent they have the same coordination sphere. So, we may suppose the formula $[\text{Sn}_3(\text{OH})_4]^{2+}$, that is a combination of three pyramids linked by joint edges in a cycle (See J.D. Donaldson et al, JCS Dalton Trans, 1995, 2273.):



The pyramidal nonplanar geometry is due to the electron pair on each tin atom.

4.3 In the acidic solution the hydrated tin(II) ions are formed, in the basic media – the anions $[\text{Sn}(\text{OH})_3]^-$, $[\text{Sn}(\text{OH})_6]^{4-}$ and oligonuclear species such as $[\text{Sn}_2\text{O}(\text{OH})_4]^{2-}$, $[\text{Sn}_4\text{O}(\text{OH})_{10}]^{4-}$.

4.4 $2 \text{BiCl}_3 + 3 \text{SnCl}_2 + 6 \text{HCl} \rightarrow 2 \text{Bi} + 3 \text{H}_2\text{SnCl}_6$

$$E^\circ = 0.317 - 0.15 = 0.167 \text{ V,}$$

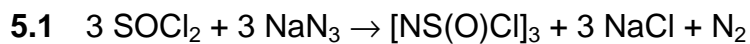
$$K = \exp\left(\frac{nFE^\circ}{RT}\right) = \exp\left(\frac{6 \times 96500 \times 0.167}{8.314 \times 298}\right) = 8.90 \times 10^{16}$$

THEORETICAL PROBLEM 5

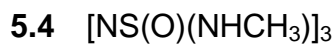
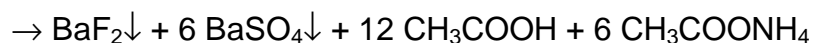
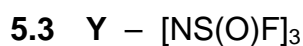
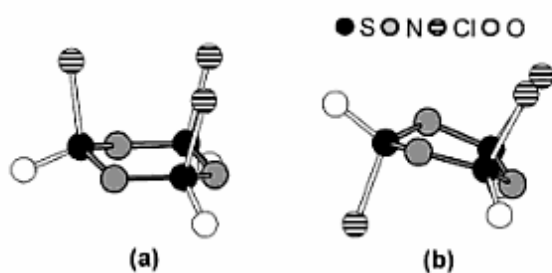
Inorganic chains and rings

- 5.1 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.
- 5.2 Draw two stereoisomers of **X**.
- 5.3 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.
- 5.4 **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.
- 5.5 Give two examples of molecules or ions which are isoelectronic to **Y**, draw their structures.
- 5.6 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.
-

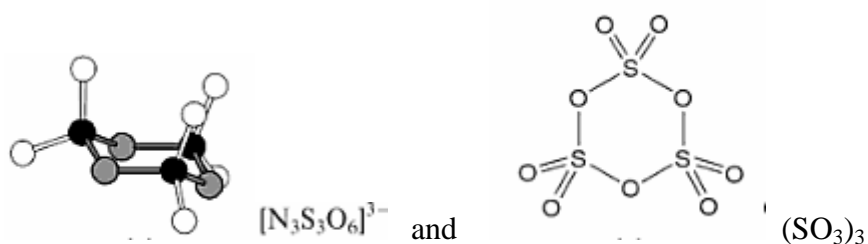
SOLUTION OF PREPARATORY PROBLEM 5



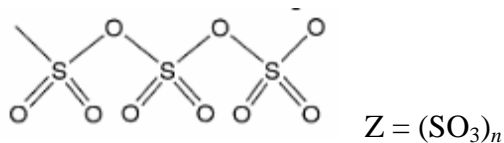
5.2



5.5



5.6



THEORETICAL 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 cm³ of 28% sodium hydroxide is triturated in a small Erlenmeyer flask with 3.5 g of finely ground Na₂SO₃ · 7 H₂O; 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 H₂O and CO₂... 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 400 °C and mixed with **C** in a such way that Na : **X** molar ratio is 3 : 1. Mixture is heated to 800 °C 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 CO₂.

A solution of 30 g of KOH in 50 cm³ of water is prepared; 10 g of **A** is added and the mixture is boiled in an open 250 cm³ 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 P₂O₅. The formed compound **E** can be recrystallized by dissolving in dil. KOH and evaporated in vacuum».

- 6.1** 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.

6.2 Write all the reaction equations.

SOLUTION OF PREPARATORY PROBLEM 6

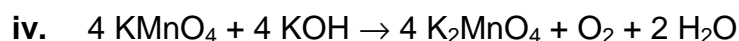
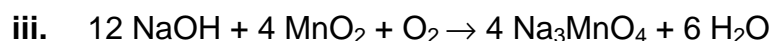
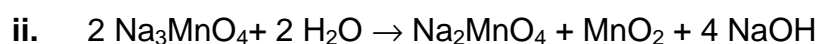
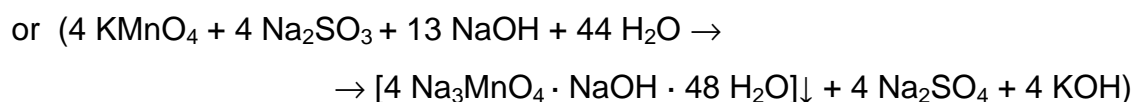
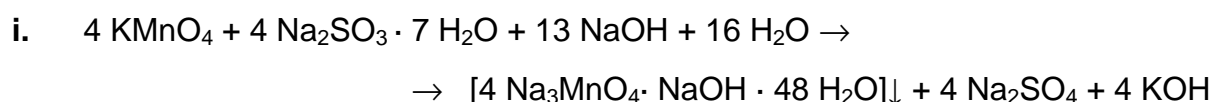
6.1 Anhydrous salt **D** is the main constituent of compound **B**. We may suppose that **B** is a hydrate of **D**. The Na : **X** molar ratio in **D** is 3 : 1. **D** is not a binary compound Na_3X as in this case $M_{\text{X}} = (29.3 \times 69 / 70.7) = 28.6$. There is no such element. So, **D** contains some other element(s) too. Oxygen is the most probable element, *i.e.*, **D** is Na_3XO_n (salt **D** cannot have formulae of $\text{Na}_3\text{H}_m\text{XO}_n$ type as all volatiles should be removed under reaction conditions used for synthesis of **D** (heating at 800 °C). High content of **X** in compound **C** allows one to suppose that **C** is a binary compound, *i.e.*, it is an oxide of **X**. Now we can determine **X**.

Oxide	X_2O	XO	X_2O_3	XO_2	X_2O_5	XO_3	X_2O_7	XO_4
M_{X}	13.74	27.48	41.22	54.96	68.70	82.43	96.17	109.91

Therefore, **X** is Mn and **C** is MnO_2 . From the content of Mn in **D** we derive its formula Na_3MnO_4 . The manganese oxidation state in this compound is V. Under heating or cooling, the alkaline solution of **D** disproportionates, giving solid MnO_2 and a green solution. Solutions of manganese(VII) derivatives are usually purple but not green. Therefore, the solution contains a salt of manganese(VI). The analogous green solution is formed in the last procedure. We may conclude that this procedure leads to manganate, K_2MnO_4 . Indeed, the content of Mn in K_2MnO_4 (compound **E**) is 27.9 %. Compound **B** (a Mn(V) derivative) is obtained by the reaction of **A** with sodium sulfite which is a well-known reducing agent. Heating of the alkaline solution of **A** affords K_2MnO_4 . It is possible only if **A** is a Mn(VII) derivative. Indeed, the Mn content in **A** corresponds to the formula of KMnO_4 . The remaining unknown compound is **B**. Above we supposed that **B** is a hydrate of **D**. Calculations using the formula of $\text{Na}_3\text{MnO}_4 \cdot n\text{H}_2\text{O}$ lead to $M_{\text{r}}(\text{B}) = 413.5$. It corresponds to $n = 12.5$. However, $M_{\text{r}}(\text{B}) = 381.2$ from the Na content. In other words, Na : Mn ratio in **B** is not 3 : 1 but 3.25 : 1.

This additional sodium appears due to the presence of some other Na compound(s) in the solvate. To determine this compound, the analysis of the synthetic procedure is required. During the synthesis of **B** solvate is washed with NaOH solution. So, the possible formula of **B** is $\text{Na}_3\text{MnO}_4 \cdot 0.25 \text{NaOH} \cdot n \text{H}_2\text{O}$. From Na and Mn content we conclude that $n = 12$. Finally, **B** is $[4 \text{Na}_3\text{MnO}_4 \cdot \text{NaOH} \cdot 48 \text{H}_2\text{O}]$.

6.2 Four reactions are discussed in the text. They are:



THEORETICAL 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.

- 7.1 Determine the equilibrium constant K_1 for the above reaction.
- 7.2 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 $K_2 = K_1 / 2$.

- 7.3 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 $\eta = n_{\text{eq}}(AB) / n_{\text{max}}(AB)$ 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.

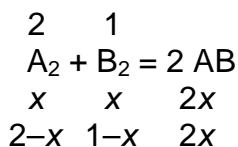
- 7.4 At what x the yield is extremal (minimal or maximal)?
- 7.5 What is the yield at: a) $x \rightarrow \infty$; b) $x \rightarrow 0$?
- 7.6 Draw the graph of $\eta(x)$.

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

- 7.7 At what x the equilibrium amount of AB is maximal?

SOLUTION OF PREPARATORY PROBLEM 7

7.1 The initial ratio $A_2 : B_2 = 2 : 1$

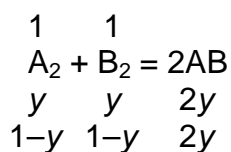


$$n(AB) = 2x = n(A_2) + n(B_2) = (2-x) + (1-x),$$

$$x = 0.75$$

$$K_1 = \frac{n(AB)^2}{n(A_2) n(B_2)} = \frac{1.5^2}{1.25 \times 0.25} = 7.2$$

7.2 The initial ratio $A_2 : B_2 = 1 : 1$



$$K_1 = \frac{n(AB)^2}{n(A_2) n(B_2)} = \frac{(2y)^2}{(1-y) \times (1-y)}$$

The ratio of heteronuclear to homonuclear molecules:

$$\frac{n(AB)}{n(A_2) + n(B_2)} = \frac{2y}{(1-y) + (1-y)} = \frac{y}{1-y} = \sqrt{\frac{K_1}{4}} = 1.34$$

7.3 New equilibrium constant: $K_2 = K_1 / 2 = 3.6$.

Equilibrium amounts: $n(AB) = 1.5$ mol, $n(A_2) = 1.25$ mol, $n(B_2) = 0.25+x$ mol,

$$K_2 = \frac{1.5^2}{1.25 \times (0.25+x)} = 3.6,$$

$x = 0.25$ mol = 25 % of initial amount of B_2 should be added.

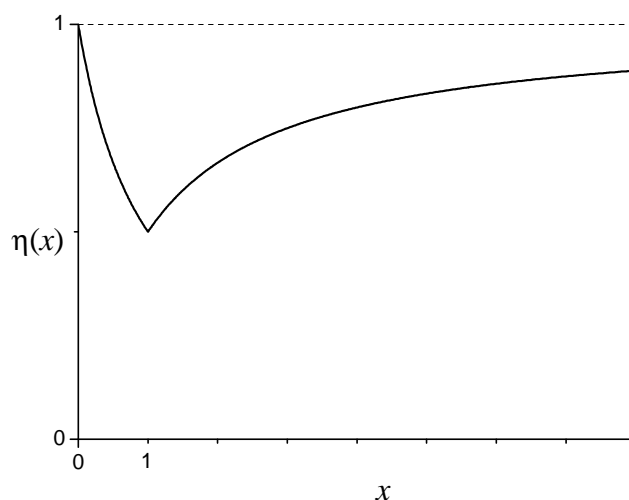
7.4 Consider two initial mixtures: $A_2 : B_2 = x : 1$ and $A_2 : B_2 = 1/x : 1 = 1 : x$. It is clear that in both cases the equilibrium yield is the same, hence $\eta(x) = \eta(1/x)$. The value $x = 1$ for such functions is the extremum point. We can prove it in the following way. Consider the identity:

$$\eta(1) - \eta(x) = \eta(1) - \eta\left(\frac{1}{x}\right)$$

near the point $x = 1$. If $\eta(x)$ is an increasing or decreasing function at $x = 1$, then near this point both sides of the identity will have opposite signs. Hence, either $\eta(x) = \text{const}$ (which is chemical nonsense), or $x = 1$ is the point of extremum.

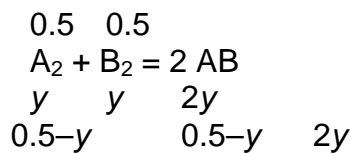
- 7.5** a) At $x \rightarrow \infty$ the very large amount of A_2 will almost completely shift the equilibrium $A_2 + B_2 = 2AB$ to the right, and almost all B_2 will be converted to AB , the yield will tend to 1, $\eta(x \rightarrow \infty) \rightarrow 1$.
- b) At $x \rightarrow 0$ ($1/x \rightarrow \infty$) the situation is the same as in (a) if we interchange A_2 and B_2 , that is $\eta(x \rightarrow 0) \rightarrow 1$.

- 7.6** It follows from question 5 that at $x = 1$ the function $\eta(x)$ has a minimum, because at $x = 0$ or $x = \infty$ it approaches the maximum possible value of 1. Qualitatively, the graph is as follows:



- 7.7** Suppose we have in total 1 mol of A_2 and B_2 , and the molar ratio $A_2 : B_2 = x : 1$. Then, the initial amounts of reagents are: $n(A_2) = x/(x+1)$, $n(B_2) = 1/(x+1)$. It follows from the symmetry between A_2 and B_2 that the equilibrium amount of AB will be the same for the molar ratios x and $1/x$, hence $x = 1$ corresponds to the maximum or minimum $n_{\text{eq}}(AB)$.

If x is very large (small), then the initial amount of B_2 (A_2) will be small and so will be $n_{\text{eq}}(\text{AB})$. Therefore, the maximum amount of AB will be obtained at $A_2 : B_2 = 1 : 1$. The equilibrium calculation for this case is as follows.



$$K = \frac{n(\text{AB})^2}{n(A_2) n(B_2)} = \frac{(2y)^2}{(0.5-y)^2}$$

$$y = \frac{\sqrt{K}}{4+2\sqrt{K}}$$

$$n_{\text{eq}}(\text{AB}) = \frac{\sqrt{K}}{2+\sqrt{K}}$$

THEORETICAL 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.

- 8.1** Write down the formula of these crystals.
- 8.2** 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.

- 8.3** By rectification of aqueous ethanol one can increase its concentration to not more than 95.5 wt.%. This is due to the fact that:
- pressures of water and ethanol vapor are the same
 - mole fractions of ethanol in the gas and liquid phases are equal
 - water forms a stable complex with ethanol
 - 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.

- 8.4** 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.

8.5 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 $p = p_{\text{sat}} \gamma x$, 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.

	$\Delta_f H_{298}^0 / (\text{kJ mol}^{-1})$	$p_{\text{sat}} / \text{Pa at 298K}$
$\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$	-2277.4	1047
$\text{CuSO}_4 \cdot 3 \text{H}_2\text{O}$	-1688.7	576
$\text{CuSO}_4 \cdot \text{H}_2\text{O}$	-1084.4	107
CuSO_4	-770.4	
$\text{H}_2\text{O}(l)$	-285.83	3200
$\text{H}_2\text{O}(g)$	-241.83	

SOLUTION OF PREPARATORY PROBLEM 8

8.1 $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$.

8.2 The Clausius-Clapeyron equation for the decomposition of a solid hydrate:



has the form:

$$\frac{dp_h}{dT} = \frac{\Delta H_d}{T \Delta V} \gg \frac{p_h \Delta H_d}{2RT^2},$$

where p_h is the vapor pressure of water over the hydrate, ΔH_d is the enthalpy of decomposition. The solution of this equation is:

$$p_h = p_{h0} \exp \left(\frac{\Delta H_d}{2R} \left(\frac{1}{T_0} - \frac{1}{T} \right) \right),$$

where $p_{h0} = 1047$ Pa is the saturated vapor pressure over $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$ and $T_0 = 298$ K. Enthalpy of decomposition of $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$ is:

$$\Delta H_d = 2 \times (-241.83) - 1688.7 + 2277.4 = 105.04 \text{ kJ mol}^{-1}.$$

The similar equation describes the temperature dependence of the vapor pressure of water p_w :

$$p_w = p_{w0} \exp\left(\frac{\Delta H_{\text{vap}}}{R} \left(\frac{1}{T_0} - \frac{1}{T}\right)\right).$$

The enthalpy of vaporization of water is: $\Delta H_{\text{vap}} = -241.83 + 285.83 = 44.0 \text{ kJ mol}^{-1}$. The humidity is the ratio of two vapor pressures:

$$\frac{p_h}{p_w} = \frac{p_{h0}}{p_{w0}} \exp\left(\frac{\Delta H_d/2 - \Delta H_{\text{vap}}}{R} \left(\frac{1}{T_0} - \frac{1}{T}\right)\right) = 0.35.$$

From this equation we find the required temperature:

$$\frac{1}{T} = \frac{1}{T_0} - \frac{R}{\Delta H_d/2 - \Delta H_{\text{vap}}} \ln \frac{0.35 p_{w0}}{p_{h0}} = \frac{1}{298} - \frac{8.314}{(105.04/2 - 44) \times 10^3} \ln \frac{0.35 \times 3200}{1047} =$$

$$= 0.00329$$

$$T_0 = \frac{1}{0.00329} = 304 \text{ K or } 31^\circ \text{C}.$$

8.3 b)

8.4 After several repetitions of the procedure, the equilibrium is established between the anhydrous copper sulfate and its monohydrate: $\text{CuSO}_4 \cdot \text{H}_2\text{O} = \text{CuSO}_4 + \text{H}_2\text{O}$. In this case the saturated vapor pressure of water over its solution in ethanol is equal to the saturated vapor pressure of water over $\text{CuSO}_4 \cdot \text{H}_2\text{O}$. Thus, $p_h = p_w \gamma x$,

$x = \frac{p_h}{p_w \gamma} = 0.0136$, the mass fraction of water is:

$$w(\text{H}_2\text{O}) = \frac{x M(\text{H}_2\text{O})}{x M(\text{H}_2\text{O}) + (1-x) M(\text{C}_2\text{H}_5\text{OH})} = 0.0054 \text{ or } 0.54 \%$$

8.5 Enthalpy of decomposition of $\text{CuSO}_4 \cdot \text{H}_2\text{O}$ is: $\Delta H_d = -241.83 - 770.4 + 1084.4 = 72.17 \text{ kJ mol}^{-1}$. From the equations above it follows that:

$$x = \frac{p_h}{p_w \gamma} = \frac{p_{h0}}{\gamma p_{w0}} \exp\left(\frac{\Delta H_d - \Delta H_{\text{vap}}}{R} \left(\frac{1}{T_0} - \frac{1}{T}\right)\right).$$

At $T = 273 \text{ K}$, $x = 0.0048$, $w = 0.19 \%$; at $T = 313 \text{ K}$ $x = 0.0235$, $w = 0.93 \%$.

THEORETICAL 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

- 9.1** *TON* is a dimensionless value. What is the dimension of *TOF*? Derive a relation between *TON* and *TOF*.
- 9.2** Let a catalytic reaction $A + \text{Cat} \rightarrow B$ 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*.

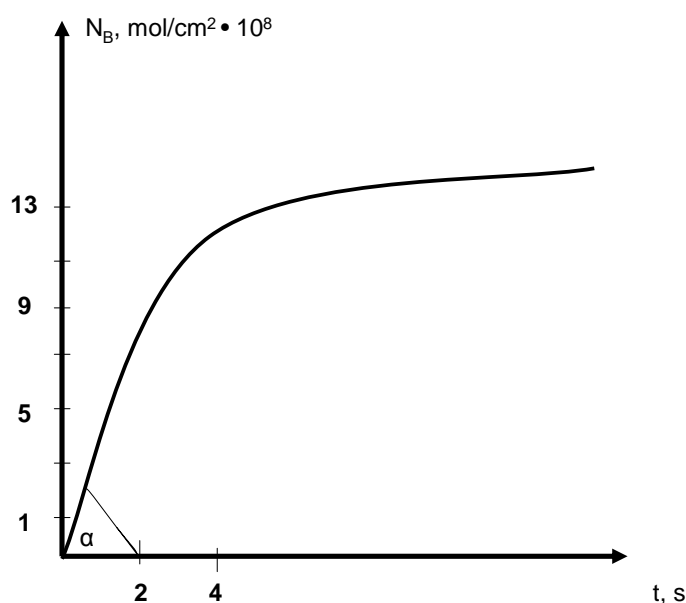


Figure 1a. The amount of the product N_B as a function of time

- 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*.

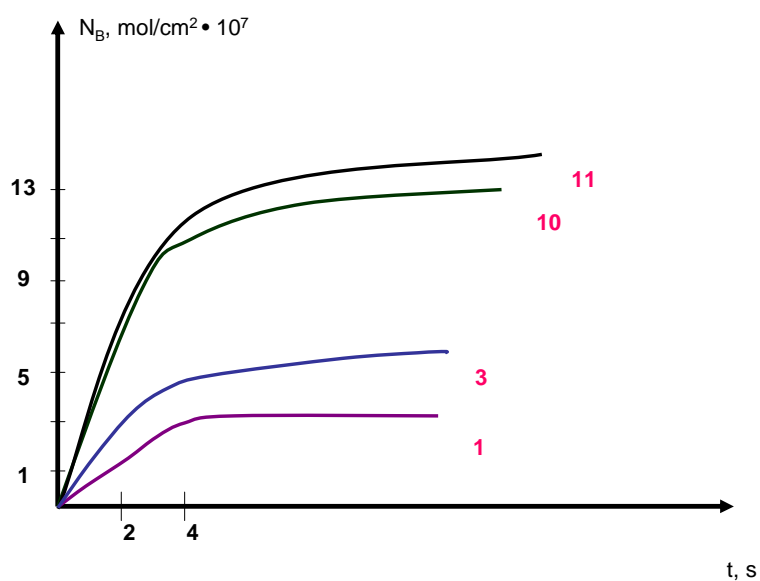


Figure 1b. The amount of the product N_B as a function of time

- 9.3 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 cm² of the surface (less than one monolayer) is shown in Fig. 2a. Calculate *TOF*.

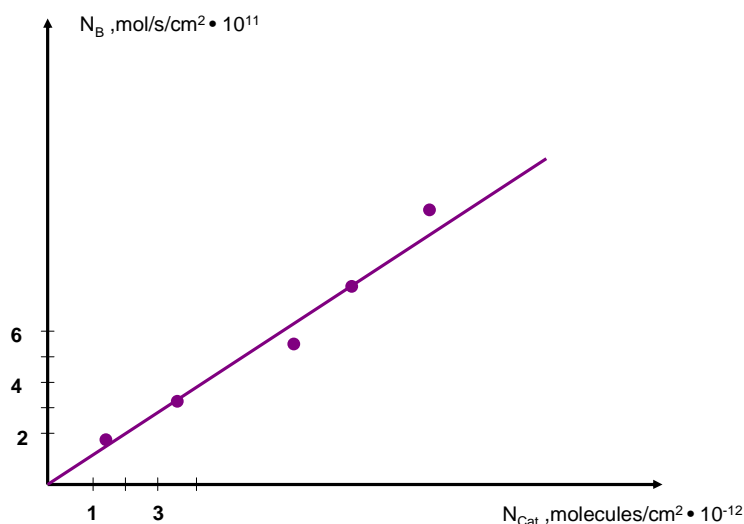
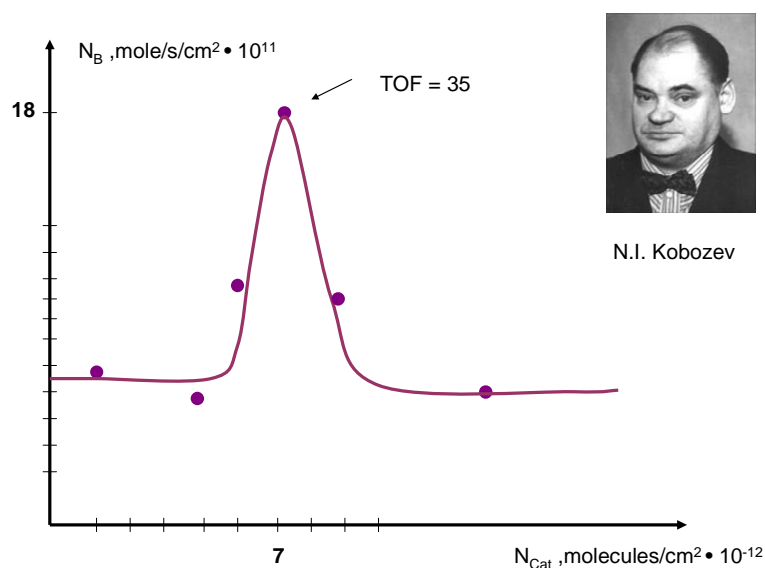


Figure 2a. The dependence of N_b on N_{Cat}

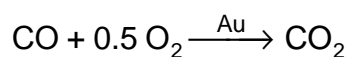
- b) Russian scientist professor Nikolay I. Kobozev has shown that the dependence of N_B on N_{Cat} can be much more complicated. The corresponding curve in Fig. 2b has maximum! According to the Kobozev's theory (a simplified version) a structure consisted of n deposited atoms rather than a single atom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

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



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

The maximum rate of reaction r_1 {mol/cm²/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, $r_2 = \frac{1}{4} r_1$.

9.4 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.

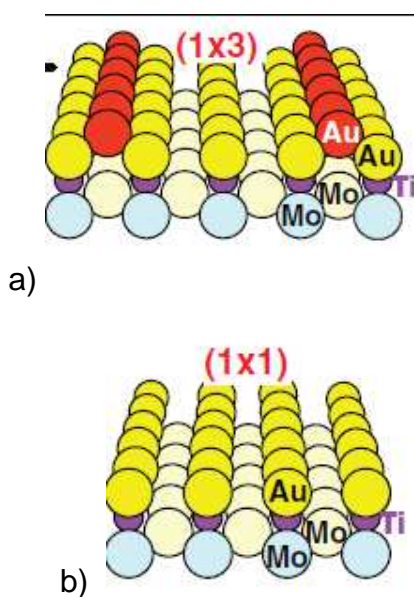


Figure 3. Structure of the gold catalyst deposited on the Mo-TiO₂ support.

a) Bilayer structure; b) monolayer structure

SOLUTION OF PREPARATORY PROBLEM 9

9.1 The *TOF* unit is {time⁻¹}. SI unit for *TOF* is {s⁻¹}.

TOF relates to *TON* by the equation

$$TOF \times t = TON,$$

where *t* is the time till the moment of inactivation of a catalyst. The formula gives the upper limit for *TON*. It assumes that the catalyst works with its best efficiency (*TOF*) all the time and becomes inactivated suddenly, in a moment. It is more realistic to assume that *TOF* goes down gradually. Then the following relation is valid:

$$TOF \times t \geq TON.$$

9.2 a) *TOF* is a maximum value of

$$\frac{\Delta N_B}{\Delta t \times 10^{15}} \quad (1)$$

Maximum of $\Delta N_B / \Delta t$ corresponds to the initial linear part of the curve in Fig. 1a and is equal to

$$\frac{\Delta N_B}{\Delta t} = \operatorname{tg} \alpha = \left(\frac{7}{2} \right) \times 10^{-8} \frac{\text{mol}}{\text{cm}^2 \text{ s}} = 21 \times 10^{15} \frac{\text{mol}}{\text{cm}^2 \text{ s}}$$

TOF is equal to

$$TOF = \frac{\Delta N_B}{\Delta t \times 10^{15}} = 21$$

b) There are several curves in Fig. 1b. It is obvious that the value of $\Delta N_B/\Delta t$ for the initial linear parts of the curves goes up with the increase of the initial pressures of the reagent A. However for curves (10) and (11) the initial slopes $\Delta N_B/\Delta t$ are the same. It means that the maximum efficiency of the catalyst is achieved. Now $\Delta N_B/\Delta t$ is independent of the reagent pressure and no more A can be converted into products per unit of time per catalytic site. The initial slopes of the curves (10) and (11) should be used to calculate *TOF* and *TON*

$$\frac{\Delta N_B}{\Delta t \times 10^{15}} = 210 \text{ s}^{-1}; \quad TON \leq TOF \times t = 210 \times 40 \times 60 = 5 \times 10^5$$

9.3 a) The slope of the linear dependence in Fig. 2a should be used to calculate *TOF*:

$$TOF = \text{tg } \alpha = 6 \text{ s}^{-1}$$

It is assumed that every single atom of the metal forms a catalytic site and works independently. *TOF* is independent of the amount of atoms deposited.

b) In this case a group of n atoms, rather than a single atom, forms a catalytic site. The number of catalytic sites is

$$k = \frac{N_B}{TOF} = \frac{18 \times 6.02 \cdot 10^{23} \times 10^{-11}}{35} = 3.1 \cdot 10^{12} \text{ mol sec} / \text{cm}^2,$$

and the number of atoms n in one catalytic site is equal to:

$$n = \frac{N_{\text{Cat}}}{k} = \frac{N_{\text{Cat}}}{N_B} TOF = \frac{7 \cdot 10^{12}}{3.1 \cdot 10^{12}} = 2.2 \approx 2$$

9.4 The authors of this study considered every single Au atom to be a catalytic site. One has to calculate the number of Au atoms involved in the catalytic process in Fig. 3a and 3b. In the case (b), all yellow spheres are taking part in the reaction. In the case (a), 1/3 of the yellow spheres from the lower monolayer are involved together with all red spheres. 2/3 of the yellow spheres are blocked by the red spheres from the top and do not participate in the catalytic reaction.

Let N_{Au} be the number of the yellow spheres in Fig. 3b. The number of the red spheres in Fig. 3a is equal to 1/3 N_{Au} . The total number of Au atoms involved in

catalytic reaction in Fig. 3a is $1/3 N_{\text{Au}}(\text{red}) + 1/3 N_{\text{Au}}(\text{yellow})$. The rate of the reaction in case (a) is:

$$r_2 = 4 r_1 = r_2(\text{red}) + \frac{1}{3} r_1,$$

where $r_2(\text{red})$ and $1/3 r_1$ are partial rates for the red and yellow spheres, respectively.

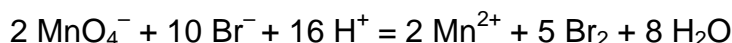
Finally,
$$\frac{\text{TOF(a)}}{\text{TOF(b)}} = \frac{4 r_1 - \frac{1}{3} r_1}{\frac{1}{3} N_{\text{Au}}} : \frac{r_1}{N_{\text{Au}}} = \frac{3(11/3 r_1)}{r_1} = 11$$

THEORETICAL 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.

10.1 Oxidation of bromide ion by permanganate in acidic solution



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

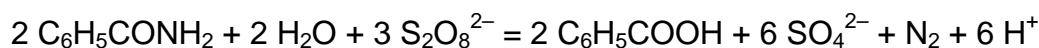
$$r = k c(\text{MnO}_4^-) c^2(\text{Br}^-) c^3(\text{H}^+)$$

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

$$r = k c(\text{MnO}_4^-) c(\text{Br}^-) c(\text{H}^+)$$

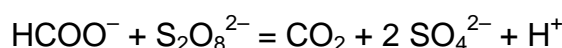
where c are the total concentrations of reactants. In both cases $c(\text{MnO}_4^-) \ll c(\text{Br}^-)$, $c(\text{H}^+)$.

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



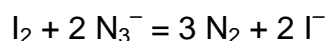
$$r = k [\text{Ag}^+] [\text{S}_2\text{O}_8^{2-}]$$

10.3 Oxidation of formate ion by peroxydisulfate in water solution



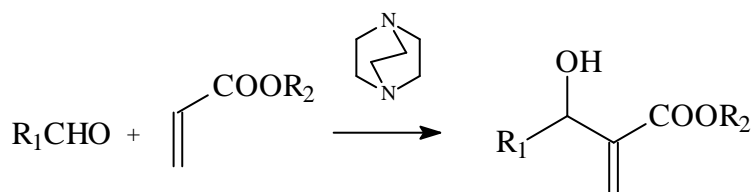
$$r = k [\text{HCOO}^-]^{1/2} [\text{S}_2\text{O}_8^{2-}]$$

10.4 Oxidation of azide ion by iodine in carbon disulfide solution



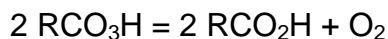
$$r = k [\text{N}_3^-]$$

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



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

10.6 Decomposition of peroxyacids in water solution



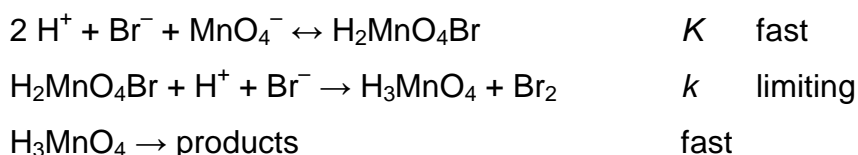
$$r = c^2(\text{RCO}_3\text{H}) \frac{k_1 [\text{H}^+]}{(k_2 + [\text{H}^+])^2},$$

where $c(\text{RCO}_3\text{H})$ is the total concentration of acid. Consider the following: when the mixture of normal $\text{RCO}-\text{O}-\text{O}-\text{H}$ and isotopically labeled $\text{RCO}-^{18}\text{O}-^{18}\text{O}-\text{H}$ peroxyacid is used as a reactant, the main species of evolving oxygen are $^{16}\text{O}_2$ and $^{18}\text{O}_2$.

SOLUTION OF PREPARATORY PROBLEM 10

Below are given the mechanisms of these reactions, established by various experimental methods. However, the limited data given in the text of a problem allow multiple mechanisms. Therefore the only two criteria for the correct solutions are: 1) the consistency of the mechanism with the rate law; 2) the chemical sense.

10.1 The schematic mechanism is:



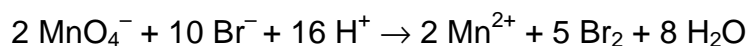
At low concentrations of proton and bromide the equilibrium of the first reaction is shifted to the left, hence the concentration of the complex $\text{H}_2\text{MnO}_4\text{Br}$ is

$$[\text{H}_2\text{MnO}_4\text{Br}] = K [\text{MnO}_4^-] [\text{Br}^-] [\text{H}^+]^2 \approx K c(\text{MnO}_4^-) c(\text{Br}^-) c^2(\text{H}^+)$$

At high concentrations of proton and bromide the equilibrium of the first reaction is shifted to the right, hence the concentration of complex $\text{H}_2\text{MnO}_4\text{Br}$ equals the total concentration of permanganate:

$$[\text{H}_2\text{MnO}_4\text{Br}] \approx c(\text{MnO}_4^-)$$

The rate of the reaction



is half of that of the rate-determining step:

$$r = \frac{1}{2} k [\text{H}_2\text{MnO}_4\text{Br}] [\text{H}^+] [\text{Br}^-]$$

in the case (a)

$$r = \frac{1}{2} k [\text{H}_2\text{MnO}_4\text{Br}] [\text{H}^+] [\text{Br}^-] \approx k_{\text{eff}} c(\text{MnO}_4^-) c^2(\text{Br}^-) c^3(\text{H}^+)$$

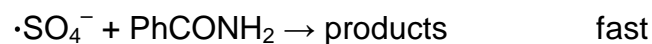
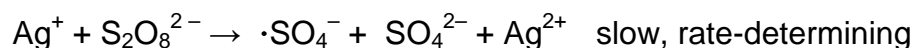
where $k_{\text{eff}} = \frac{1}{2} k K$.

In the case (b)

$$r = \frac{1}{2} k [\text{H}_2\text{MnO}_4\text{Br}] [\text{H}^+] [\text{Br}^-] \approx k_{\text{eff}} c(\text{MnO}_4^-) c(\text{Br}^-) c(\text{H}^+)$$

where $k_{\text{eff}} = \frac{1}{2} k$.

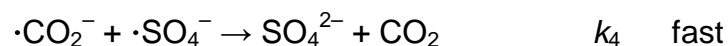
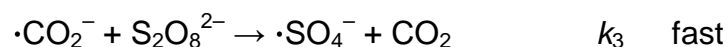
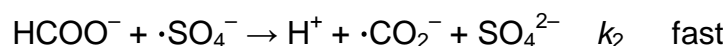
10.2 The catalytic effect of silver is due to formation of silver(II) ions and sulfate ion radicals upon reaction of Ag^+ with persulfate. The mechanism is:



The first reaction is the rate-determining step; therefore the overall oxidation reaction has the same order as the rate-determining step:

$$r = k [\text{Ag}^+] [\text{S}_2\text{O}_8^{2-}]$$

10.3 The minimal mechanism includes the following steps:



The second and the third reaction make a chain process involving consumption of peroxydisulfate and formate. The first reaction is very slow, so most of peroxydisulfate is consumed in the third reaction. Applying the steady-state approximation to $\cdot\text{SO}_4^-$ and $\cdot\text{CO}_2^-$ we get:

$$2 r_1 - r_2 + r_3 - r_4 = 0$$

$$r_2 - r_3 - r_4 = 0$$

Hence

$$r_1 = r_4$$

$$r_2 - r_3 = r_1$$

Since the rate of the first reaction is very low, then

$$r_1 = r_4$$

$$r_2 = r_3$$

Applying the rate laws we get:

$$k_1 [\text{S}_2\text{O}_8^{2-}] = k_4 [\cdot\text{CO}_2^-] [\cdot\text{SO}_4^-]$$

$$k_2 [\text{HCOO}^-] [\cdot\text{SO}_4^-] = k_3 [\cdot\text{CO}_2^-] [\text{S}_2\text{O}_8^{2-}]$$

Hence

$$[\cdot\text{CO}_2^-] = (k_1 k_2)^{1/2} (k_3 k_4)^{-1/2} [\text{HCOO}^-]^{1/2}$$

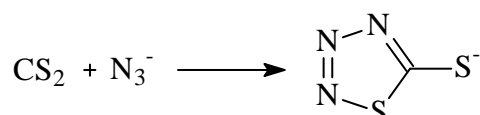
$$[\cdot\text{SO}_4^-] = (k_1 k_3)^{1/2} (k_2 k_4)^{-1/2} [\text{S}_2\text{O}_8^{2-}] [\text{HCOO}^-]^{-1/2}$$

The rate of the reaction is equal to the rate of formate consumption:

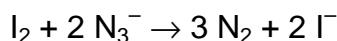
$$\begin{aligned} r = r_2 = k_2 [\text{HCOO}^-] [\cdot\text{SO}_4^-] &= (k_1 k_2 k_3)^{1/2} k_4^{-1/2} [\text{HCOO}^-]^{1/2} [\text{S}_2\text{O}_8^{2-}] = \\ &= k_{\text{eff}} [\text{HCOO}^-]^{1/2} [\text{S}_2\text{O}_8^{2-}] \end{aligned}$$

A more complex mechanism includes the formation of OH radicals and several chain termination reactions. That's why the given rate law is valid only for a limited range of reactant concentrations.

10.4 The rate-determining step is the addition of azide ion to the solvent, carbon disulfide:



The oxidation of this ion by iodine is a series of fast reactions. The overall rate of the reaction



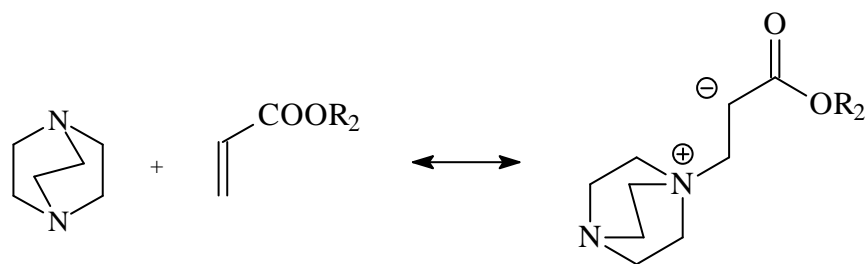
is half of that of the azide-CS₂ reaction:

$$r = \frac{1}{2} k [\text{N}_3^-] [\text{CS}_2].$$

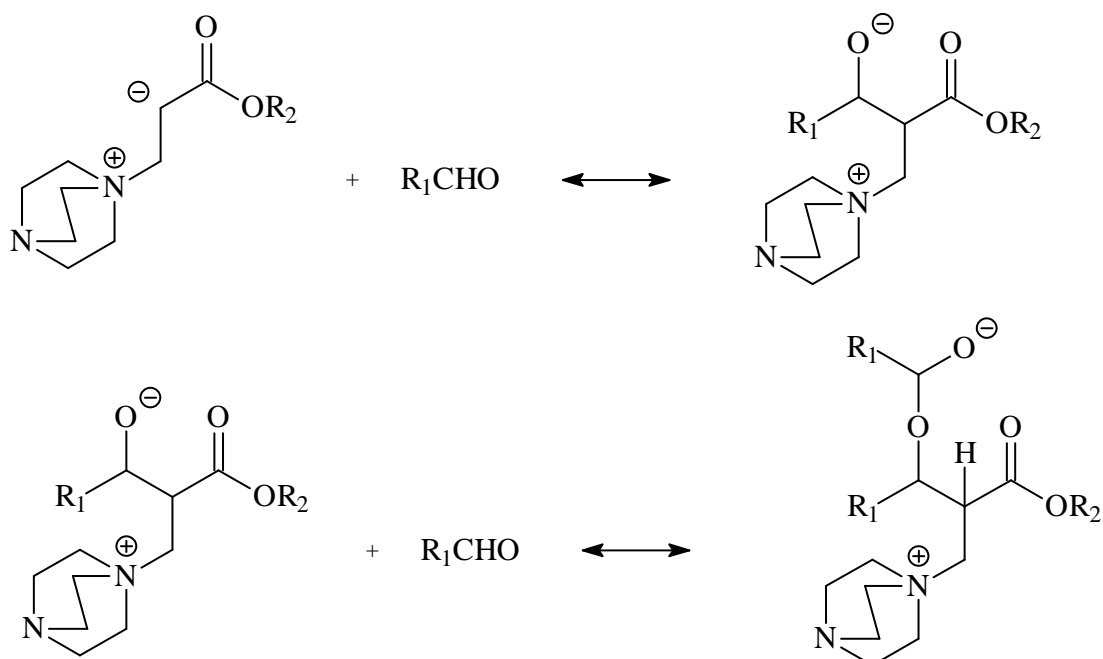
Introducing the effective constant $k_{\text{eff}} = \frac{1}{2} k [\text{CS}_2]$ we get:

$$r = k_{\text{eff}} [\text{N}_3^-].$$

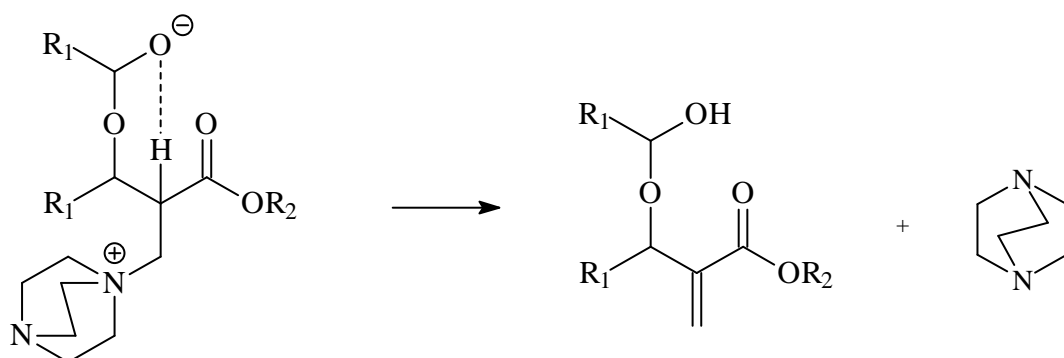
10.5 The reaction mechanism includes several steps. The first step is the reversible addition of DABCO to ether:



The next two steps are the reversible additions of two molecules of aldehyde to the zwitter ion formed in the previous step:



The rate-determining step is an intramolecular proton transfer followed by the elimination of DABCO:

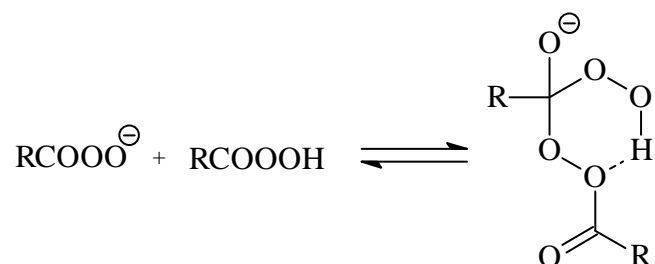


After that, the product rapidly eliminates one molecule of aldehyde. Applying quasi-equilibrium conditions to the first three steps, we get:

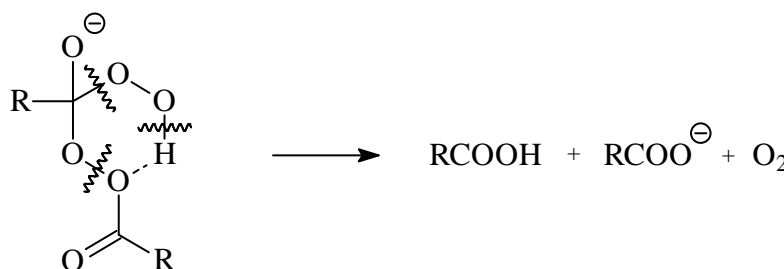
$$r = k_{\text{RDS}} K_1 K_2 K_3 [\text{aldehyde}]^2 [\text{ether}] [\text{DABCO}] = k_{\text{eff}} [\text{aldehyde}]^2 [\text{ether}] [\text{DABCO}]$$

It is worth mentioning that in protic solvents the rate-determining step is the solvent-assisted proton transfer in DABCO-ether-aldehyde adduct, hence the reaction order is one with respect to either aldehyde, or ether or base.

10.6 The first step of the reaction is the reversible addition of peroxyacid anion to the carboxylic group of peroxyacid:



The next, rate-determining step is a decomposition of the addition product:



Applying a quasi-equilibrium approximation, we get:

$$r = k_{\text{eff}} [\text{RCO}_3\text{H}] [\text{RCO}_3^-]$$

The concentrations of peroxyacid and its anion are related to the total concentration of peroxy compound $c(\text{RCO}_3\text{H})$ and proton concentration $[\text{H}^+]$ as follows:

$$[\text{RCO}_3\text{H}] = \frac{[\text{H}^+]}{K_a + [\text{H}^+]} c(\text{RCO}_3\text{H}) \quad [\text{RCO}_3^-] = \frac{K_a}{K_a + [\text{H}^+]} c(\text{RCO}_3\text{H}),$$

where K_a is the acidity constant of peroxyacid. Substituting these concentrations to the rate law we obtain:

$$r = c^2(\text{RCO}_3\text{H}) \frac{k_{\text{eff}} K_a [\text{H}^+]}{(K_a + [\text{H}^+])^2} = c^2(\text{RCO}_3\text{H}) \frac{k_1 [\text{H}^+]}{(k_2 + [\text{H}^+])^2}$$

Note that at given $c(\text{RCO}_3\text{H})$ the reaction rate is maximum if $[\text{RCO}_3\text{H}] = [\text{RCO}_3^-]$ (and $[\text{H}^+] = K_a$).

THEORETICAL 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

11.1 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.

SOLUTION OF PREPARATORY PROBLEM 11

Since this is a reactor with ideal stirring, the concentrations of substances in the output flow are equal to the concentrations inside the reactor. In a stationary state, the concentrations and quantities of substances in the reactor are constant. Consider the material balance with respect to X, Y and P.

Stationary conditions are:

$$\frac{\Delta v_{X,R}}{\Delta t} = 0 \quad \frac{\Delta v_{Y,R}}{\Delta t} = 0 \quad \frac{\Delta v_{P,R}}{\Delta t} = 0, \quad (1)$$

where $\Delta v_{X,R}$, $\Delta v_{Y,R}$, $\Delta v_{P,R}$ are the changes of the quantities for the substances X, Y and P in the reactor during time Δt . The quantity of the substance in the reactor may change due to input flow, chemical reaction, and output flow:

$$\frac{\Delta v_{X,R}}{\Delta t} = \left(\frac{\Delta v_{X,R}}{\Delta t} \right)_{\text{input}} + \left(\frac{\Delta v_{X,R}}{\Delta t} \right)_{\text{reaction}} + \left(\frac{\Delta v_{X,R}}{\Delta t} \right)_{\text{output}} \quad (2)$$

The same is true for Y and P.

Input flow rates of the substances are

$$\left(\frac{\Delta v_{X,R}}{\Delta t} \right)_{\text{input}} = f_X c_{X,I} \left(\frac{\Delta v_{Y,R}}{\Delta t} \right)_{\text{input}} = f_Y c_{Y,I} \left(\frac{\Delta v_{P,R}}{\Delta t} \right)_{\text{input}} = 0, \quad (3)$$

where f_X and f_Y are the input volumetric flows of the solutions of X and Y, $c_{X,I}$ and $c_{Y,I}$ – concentrations of X and Y in the respective solutions.

Let the balanced reaction equation be



where n_X , n_Y and n_P are the stoichiometric coefficients for the corresponding substances. Due to a chemical reaction the quantities of the substances in the reactor change with the rates

$$\left(\frac{\Delta v_{X,R}}{\Delta t} \right)_{\text{reaction}} = -n_X r V_R \quad \left(\frac{\Delta v_{Y,R}}{\Delta t} \right)_{\text{reaction}} = -n_Y r V_R \quad \left(\frac{\Delta v_{P,R}}{\Delta t} \right)_{\text{reaction}} = n_P r V_R, \quad (4)$$

where r – the reaction rate, V_R – the reactor volume.

The output flows of the substances are:

$$\left(\frac{\Delta v_{X,R}}{\Delta t} \right)_{\text{output}} = f_O c_{X,R} \quad \left(\frac{\Delta v_{Y,R}}{\Delta t} \right)_{\text{output}} = f_O c_{Y,R} \quad \left(\frac{\Delta v_{P,R}}{\Delta t} \right)_{\text{output}} = f_O c_{P,R}, \quad (5)$$

where f_O is the volumetric output flow, $c_{X,R}$, $c_{Y,R}$ and $c_{P,R}$ – the concentrations of substances X, Y and P in the reactor. Since the process is stationary and the reaction proceeds in the liquid phase, the output volumetric flow equals the sum of input volumetric flows:

$$f_O = f_X + f_Y \quad (6)$$

Thus the material balance equations (2) considering expressions (1) and (3)-(6) are

$$\frac{\Delta v_{X,R}}{\Delta t} = f_X c_{X,I} - n_X r V_R - c_{X,R} (f_X + f_Y) = 0$$

$$\frac{\Delta v_{Y,R}}{\Delta t} = f_Y c_{Y,I} - n_Y r V_R - c_{Y,R} (f_X + f_Y) = 0$$

$$\frac{\Delta v_{P,R}}{\Delta t} = n_P r V_R - c_{P,R} (f_X + f_Y) = 0$$

Hence

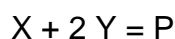
$$n_X r V_R = f_X c_{X,I} - c_{X,R} (f_X + f_Y)$$

$$n_Y r V_R = f_Y c_{Y,I} - c_{Y,R} (f_X + f_Y)$$

$$n_P r V_R = c_{P,R} (f_X + f_Y)$$

Exp. no.	$n_X r V_R$, mol/s	$n_Y r V_R$, mol/s	$n_P r V_R$, mol/s	$n_X:n_Y:n_P$
1	10.02	20.04	10.02	1:2:1
2	10.04	20.07	10.05	1:2:1
3	15.73	31.47	15.72	1:2:1
4	19.68	39.34	19.68	1:2:1

Hence the balanced reaction equation is



Now consider the rate dependence on concentrations

Exp. no.	$c_{X,R}$, mol/m ³	$c_{Y,R}$, mol/m ³	$c_{P,R}$, mol/m ³	$r V_R$, mol/s
1	299	48.2	501	10.02
2	732	30.9	335	10.04
3	8.87	351	524	15.73
4	308	66.6	492	19.68

The rate law is

$$r = k c_{X,R}^x c_{Y,R}^y c_{P,R}^p$$

or, after multiplying by reactor volume,

$$r V_R = k V_R c_{X,R}^x c_{Y,R}^y c_{P,R}^p$$

Take the logarithm of both parts of the equation

$$\ln(r V_R) = \ln(k V_R) + x \ln c_{X,R} + y \ln c_{Y,R} + p \ln c_{P,R} \quad (7)$$

The coefficients in this equation are given in the table below:

Exp. no.	$\ln c_{X,R}$	$\ln c_{Y,R}$	$\ln c_{P,R}$	$\ln (rV_R)$
1	5.70	3.88	6.22	2.30
2	6.60	3.43	5.81	2.31
3	2.18	5.86	6.26	2.76
4	5.73	4.20	6.20	2.98

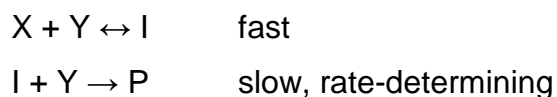
Solving the system of equations (7) we get:

$$x = 1.00 \quad y = 2.00 \quad p = 0.01 \quad \ln(kV_R) = -11.20$$

Hence the orders of the reaction are one in X, two in Y, and zero in P. The product kV_R is:

$$k V_R = \exp(-11.20) = 1.37 \cdot 10^{-5} \text{ m}^9 \text{ mol}^{-2} \text{ s}^{-1}.$$

One of the possible mechanisms that match the obtained rate law is:



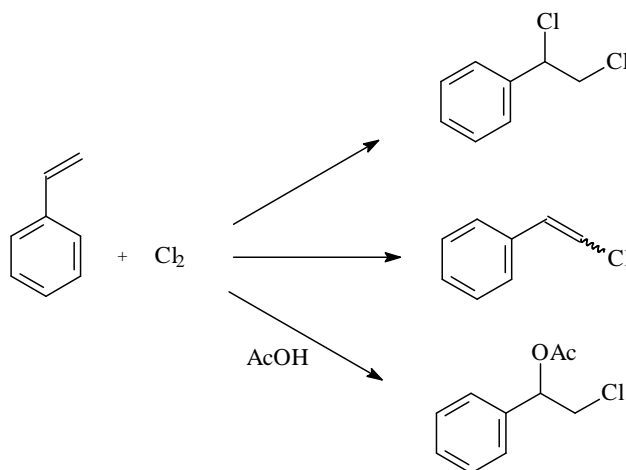
Summarizing, the obtained results are:

- ◆ the reaction equation: $X + 2 Y = P$;
- ◆ the reaction orders: 1, 2, and 0 with respect to X, Y, and P respectively;
- ◆ the product of the rate constant and reactor volume: $k V_R = 1.37 \cdot 10^{-5} \text{ m}^9 \cdot \text{mol}^{-2} \cdot \text{s}^{-1}$.

THEORETICAL PROBLEM 12

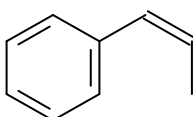
Chlorination of styrenes

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°C is given in the Table.

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

12.1 The rate constant of the overall reaction is $1.45 \cdot 10^4 \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}$ at 25 °C. What are the rate constants for the formation of 1,2-dichloro and 1-acetoxy-2-chloro adducts and 2-chlorostyrene?

12.2 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?

SOLUTION OF PREPARATORY PROBLEM 12

12.1 Since all reaction pathways obey the same rate law, the quantity of the product is proportional to the respective rate constant. The overall constant equals the sum of constants for different pathways. Hence

for 1,2-dichloro:

$$k = 1.45 \cdot 10^4 \frac{61\%}{100\%} = 8.8 \cdot 10^3 \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}$$

for 1-acetoxy-2-dichloro:

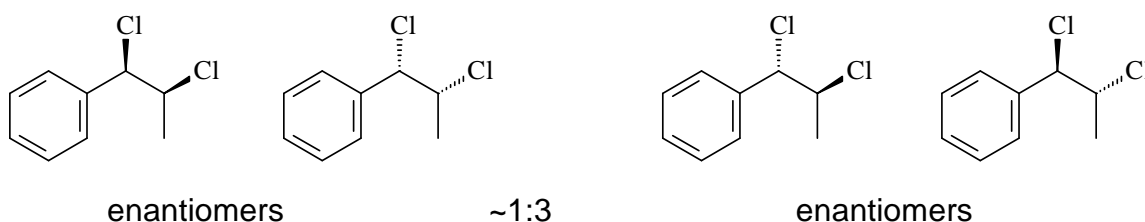
$$k = 1.45 \cdot 10^4 \frac{30\%}{100\%} = 4.4 \cdot 10^3 \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}$$

for 2-chlorostyrene:

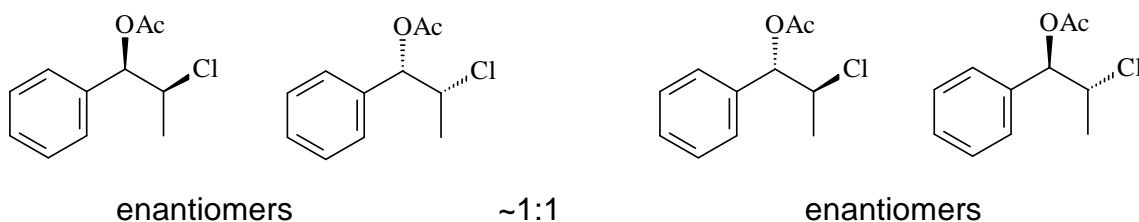
$$k = 1.45 \cdot 10^4 \frac{9\%}{100\%} = 1.3 \cdot 10^3 \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}$$

12.2 This reaction is not stereospecific and leads to the formation of diastereomeric addition products in comparable amounts. The following products are obtained (approximate ratios of product quantities at 25°C are given as an illustration):

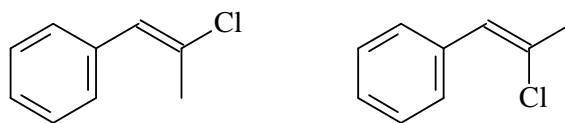
1,2-dichloro:



1-acetoxy-2-chloro:



2-chlorostyrene:



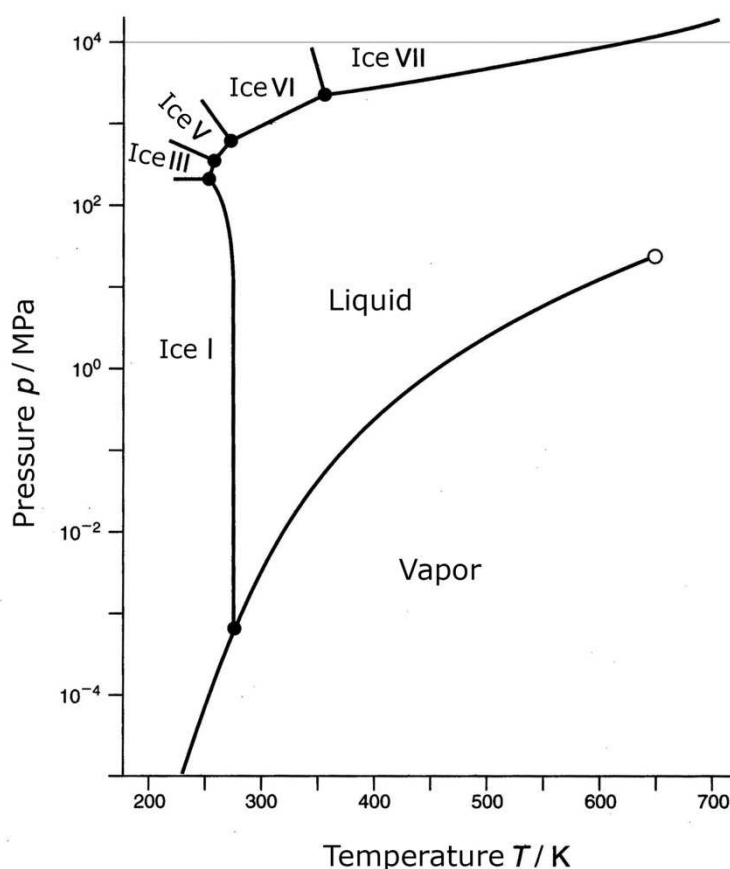
~1:3

The achiral sorbent is unable to separate enantiomers, so only 6 different fractions can be obtained. The chiral sorbent allows full separation, so in this case the determined number of products is 10.

THEORETICAL 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.

- 13.1** 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.
- 13.2** 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 ?

- 13.3** 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.
- 13.4** 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?
- 13.5** 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.
- 13.6** 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;

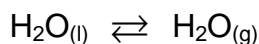
Enthalpy of fusion of ordinary ice: +6010 J mol⁻¹;

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

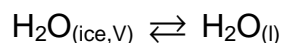
Hint. Assume that the densities of condensed phases and the enthalpies of phase transitions do not vary with pressure and temperature.

SOLUTION OF PREPARATORY PROBLEM 13

- 13.1** The boiling point of water and the melting point of ice V increase, and the melting point of ordinary ice decreases with the increasing pressure. This can be easily explained using the Le Chatelier principle. In the phase transitions

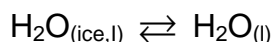


and



the volume increases and heat is absorbed ($\Delta V > 0$, $\Delta H > 0$). Hence, with the increasing pressure both equilibria are shifted to the left; consequently, temperature should be increased to keep the equilibria.

In the phase transition



the volume decreases and heat is absorbed ($\Delta V < 0$, $\Delta H > 0$). Hence, with the increasing pressure the phase equilibrium is shifted to the right, and temperature should be decreased to keep the equilibrium.

- 13.2** a) 250 K: vapor \rightarrow ice I \rightarrow ice III \rightarrow ice V \rightarrow ice VI
 b) 400 K: vapor \rightarrow liquid \rightarrow ice VII
 c) 700 K: only vapor (at high pressure it may be called "supercritical fluid"), no phase transitions occur.

13.3 Phase transitions between condensed phases are described by the Clapeyron equation:

$$\frac{dp}{dT} = \frac{\Delta H}{T \Delta V},$$

or, in approximate form:

$$\frac{\Delta p}{\Delta T} = \frac{\Delta H}{T \Delta V}.$$

We calculate the right side of this equation for the ice I \rightleftharpoons water transition. The volume change is determined from the densities:

$$\Delta V = V(\text{water}) - V(\text{ice}) = \frac{M(\text{H}_2\text{O})}{\rho(\text{water})} - \frac{M(\text{H}_2\text{O})}{\rho(\text{ice})} = \frac{18}{1.000} - \frac{18}{0.917} = -1.63 \text{ cm}^3 \text{ mol}^{-1}$$

$$\frac{\Delta p}{\Delta T} = \frac{\Delta H}{T \Delta V} = \frac{6010 \text{ J mol}^{-1}}{273 \text{ K} \times (-1.63 \cdot 10^{-6} \text{ m}^3 \text{ mol}^{-1})} = -1.35 \cdot 10^7 \text{ Pa K}^{-1} = -13.5 \text{ MPa K}^{-1}.$$

If this slope does not depend on pressure and temperature then at the pressure of 210 MPa the temperature of liquid water in equilibrium with ice I and Ice III is approximately:

$$T = 273 + \Delta T = 273 + \frac{210 - 0.1}{-13.5} = -257.5 \text{ K} = -15.5 \text{ }^\circ\text{C}.$$

This is an estimate; the real value is $-22 \text{ }^\circ\text{C}$. The difference between the estimated and real values is due to the fact that the enthalpy of fusion and densities vary with pressure. For example, at 210 MPa the enthalpy of fusion of ice I is 4230 J/mol (instead of 6010 at normal pressure), and the volume change is $\Delta V = -2.43 \text{ cm}^3/\text{mol}$ (instead of $-1.63 \text{ cm}^3 \text{ mol}^{-1}$ at normal pressure).

13.4 From the Clapeyron equation it follows that the slope of the $p(T)$ dependencies for the melting points of ice III to ice VII is determined by ΔH , T , and ΔV . The first quantity is assumed to be the same for all transitions, the temperature is comparable in all cases, hence the main contribution to the slope comes from ΔV . For ice VII, the slope is the smallest, hence, the $\Delta V = V(\text{water}) - V(\text{ice})$ is the largest, whereas $V(\text{ice})$ is the smallest. It means that ice VII is the densest form of ice (among those forms that are shown on the phase diagram).

From the phase diagram we see that the melting point of ice VII at a pressure of 10 GPa is about 630 K. This is, indeed, a very "hot" ice.

13.5 Determine the molar volume of ice VII. One mole contains $N_A/2$ cubic unit cells:

$$V_m = \frac{N_A}{2} d^3 = 3.01 \cdot 10^{23} \times (0.335 \cdot 10^{-7})^3 = 11.3 \text{ cm}^3 \text{ mol}^{-1}.$$

The density of ice VII is:

$$\rho = M / V_m = 18 / 11.3 = 1.59 \text{ g cm}^{-3}.$$

13.6 Knowing the density of ice VII, we use the Clapeyron equation to estimate its enthalpy of fusion. Comparing the triple point "water – ice VI – ice VII" and the melting point of ice VII at pressure 10 GPa we estimate the slope: $\Delta p / \Delta T = (10^4 - 2200) / (630 - 355) = 28 \text{ MPa} / \text{K}$. The volume change during melting is: $\Delta V = (18/1.00) - 11.3 = 6.7 \text{ cm}^3 \text{ mol}^{-1}$. Substituting these data into the Clapeyron equation, we get:

$$\Delta H = T \Delta V \frac{\Delta p}{\Delta T} = 355 \text{ K} \times 6.7 \cdot 10^{-6} \text{ m}^3 \text{ mol}^{-1} \times 28 \cdot 10^6 \text{ Pa K}^{-1} = 66\,000 \text{ J mol}^{-1}.$$

This value is by an order of magnitude larger than the exact value 6400 J mol^{-1} . The reason is probably due to a low resolution of the phase diagram at high pressures, which leads to a rough estimate of the slope. This result also shows that the approximations used are not valid at high pressures and temperatures.

THEORETICAL PROBLEM 14

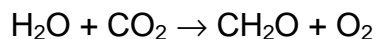
Redox reactions in photosynthesis

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 concentration of 1 mol dm⁻³ 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):



In this process water is oxidized to O₂, 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.

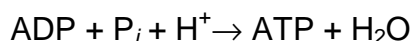
Half-reaction	Standard redox potential, E° (V)
$\text{O}_2 + 4 \text{H}^+ + 4 \text{e} \rightarrow 2 \text{H}_2\text{O}$	1.23
$\text{S} + 2 \text{H}^+ + 2 \text{e} \rightarrow \text{H}_2\text{S}$	0.14
Plastoquinone + 2 H ⁺ + 2 e → Plastoquinone·H ₂	0.52
Cytochrome f(Fe ³⁺) + e → Cytochrome f(Fe ²⁺)	0.365
$\text{NADP}^+ + \text{H}^+ + 2 \text{e} \rightarrow \text{NADP}\cdot\text{H}$	-0.11
$\text{P680}^+ + \text{e} \rightarrow \text{P680}$	1.10
$\text{Chlorophyll}^+ + \text{e} \rightarrow \text{Chlorophyll}$	0.78

- 14.1** Calculate the standard biochemical redox potential for all half-reactions presented in the table above.
- 14.2** 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 .

- 14.3** 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 $\text{NADP}\cdot\text{H}$, 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:



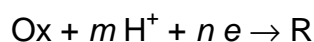
- 14.4** Write the overall reaction of light stages of photosynthesis in green plants. During light stages, light energy is converted into chemical energy stored in ATP and $\text{NADH}\cdot\text{H}$ and wasted further in dark reactions, which are highly endoergic.
- 14.5** Calculate the Gibbs energy of the overall reaction describing light stages of photosynthesis given that the standard biochemical Gibbs energy for ATP formation is $+30.5 \text{ kJ mol}^{-1}$. 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.
- 14.6** 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.

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

SOLUTION OF PREPARATORY PROBLEM 14

- 14.1** Applying the Nernst equation for a half-reaction

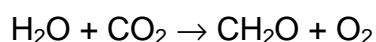


and putting $[\text{H}^+] = 10^{-7}$, we get a standard biochemical redox potential:

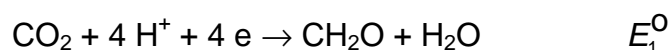
$$E^{\circ\prime} = E^{\circ} + \frac{0.0591}{n} \lg(10^{-7})^m = E^{\circ} - 0.414 \frac{m}{n}$$

Half-reaction	E° (V)	$E^{\circ\prime}$ (V)
$\text{O}_2 + 4 \text{H}^+ + 4 e \rightarrow 2 \text{H}_2\text{O}$	1.23	0.82
$\text{S} + 2 \text{H}^+ + 2 e \rightarrow \text{H}_2\text{S}$	0.14	-0.27
$\text{Plastoquinone} + 2 \text{H}^+ + 2 e \rightarrow \text{Plastoquinone} \cdot \text{H}_2$	0.52	0.11
$\text{Cytochrome f}(\text{Fe}^{3+}) + e \rightarrow \text{Cytochrome f}(\text{Fe}^{2+})$	0.365	0.365
$\text{NADP}^+ + \text{H}^+ + 2 e \rightarrow \text{NADP} \cdot \text{H}$	-0.11	-0.32
$\text{P680}^+ + e \rightarrow \text{P680}$	1.10	1.10
$\text{Chlorophyll}^+ + e \rightarrow \text{Chlorophyll}$	0.78	0.78

- 14.2** The standard electromotive force for the reaction



is the difference between standard redox potentials for oxidant and reductant.



For this reaction, the standard Gibbs energy is $480.5 \text{ kJ mol}^{-1}$, and 4 electrons are transferred from H_2O to CO_2 . Hence, the standard emf is:

$$E^{\circ} = -\frac{\Delta G^{\circ}}{nF} = -\frac{480500}{4 \times 96500} = -1.24 \text{ V} = E_1^{\circ} - 1.23 \text{ V}$$

For CO_2 reduction to carbohydrates the standard redox potential is $E_1^{\circ} = -0.01 \text{ V}$.

The standard biochemical potential is: $E_1^{\circ\phi} = -0.01 - 0.414 \frac{4}{4} = -0.42 \text{ V}$.



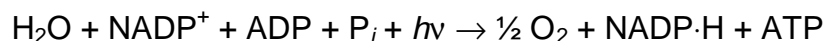
Standard Gibbs energy: $\Delta G^{\circ} = -nFE^{\circ} = -4 \times 96500 \times (-0.15) \times 10^{-3} = 57.9 \text{ kJ mol}^{-1}$.

Energy of light with wavelength 840 nm:

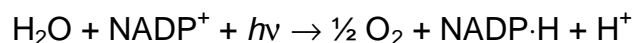
$$E_m = \frac{hcN_A}{\lambda} = \frac{6.63 \cdot 10^{-34} \times 3.00 \cdot 10^8 \times 6.02 \cdot 10^{23}}{840 \cdot 10^{-9}} \times 10^{-3} = 143 \text{ kJ mol}^{-1}$$

One quantum gives enough energy to oxidize two molecules of H_2S .

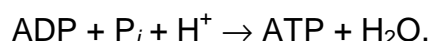
14.4 Both NADP^+ reduction and ATP formation require one proton, and during H_2O oxidation two protons are released. Hence, the overall reaction equation of light stages is:



14.5 The overall reaction is the sum of two reactions:



and



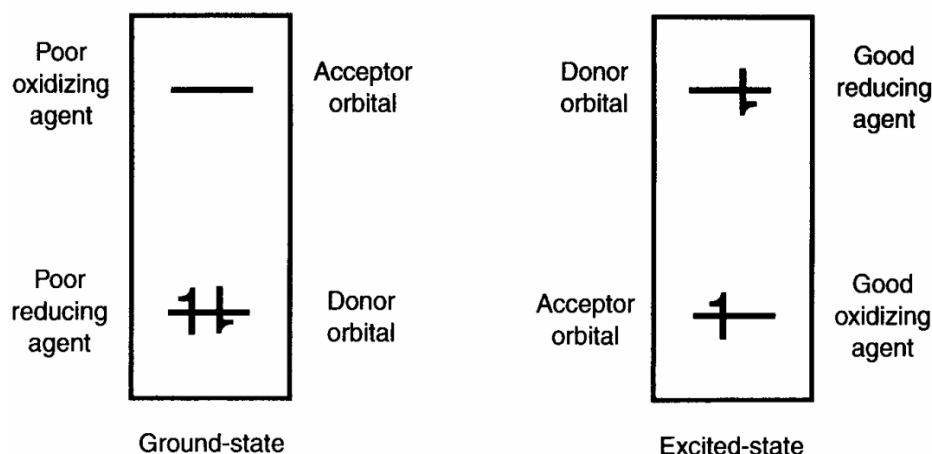
For the latter, the standard biochemical Gibbs energy is known (30.5 kJ mol^{-1}) and for the former it can be determined from the standard biochemical redox potentials.

$$\Delta G^{\circ\phi} = -nFE^{\circ\phi} = -2 \times 96500 \times (0.82 - (-0.32)) \times 10^{-3} = 220 \text{ kJ mol}^{-1}$$

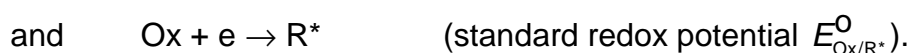
The overall light stages reaction contains no protons, hence the standard Gibbs energy is the same as the standard biochemical Gibbs energy:

$$\Delta G^{\circ} = \Delta G^{\circ\phi} = 220 + 30.5 = 250.5 \text{ kJ mol}^{-1}$$

14.6 This effect is easily understood using a simple orbital diagram (see Appendix in “Molecular Mechanisms of Photosynthesis” by R.E.Blankenship). In the ground state, a lost electron comes from the low-energy HOMO, while an acquired electron enters the high-energy LUMO. As a result, the molecule is neither a strong oxidant nor a good reductant. In the excited state, the situation is different: a lost electron leaves the high-energy LUMO, and the acquired electron comes to low-energy HOMO: both processes are energetically favorable, and the molecule can act both as a strong oxidant and a powerful reductant.



14.7 Consider two half-reactions:



The difference in their Gibbs energies is equal to the excitation energy:

$$F(E_{\text{Ox/R}}^{\circ} - E_{\text{Ox/R}^*}^{\circ}) = E_{\text{ex}} = \frac{hcN_{\text{A}}}{\lambda},$$

whence it follows:

$$E_{\text{Ox/R}^*}^{\circ} = E_{\text{Ox/R}}^{\circ} - \frac{hcN_{\text{A}}}{\lambda F}.$$

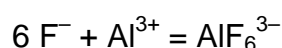
$$\text{For P680}^+: E_{\text{P680}^+/\text{P680}^*}^0 = 1.10 - \frac{6.63 \cdot 10^{-34} \times 3.00 \cdot 10^8 \times 6.02 \cdot 10^{23}}{680 \cdot 10^{-9} \times 96\,500} = -0.72 \text{ V}$$

$$\text{For Chlorophyll}^+: E_{\text{Chl}^+/\text{Chl}^*}^0 = 0.78 - \frac{6.63 \cdot 10^{-34} \times 3.00 \cdot 10^8 \times 6.02 \cdot 10^{23}}{680 \cdot 10^{-9} \times 96\,500} = -1.04 \text{ V}$$

THEORETICAL 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):



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 AlCl₃ solution ($c = 0.15 \text{ mol dm}^{-3}$) until yellow color of the indicator turned pink.

- 15.1 What process occurred at the endpoint?
- 15.2 Why heating increased the endpoint sharpness?
- 15.3 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 solution of AlCl₃ (0.1000 mol dm⁻³) in the presence of methyl red. The endpoint was attained with 10.25 cm³ of the titrant.

- 15.4 What operation (absolutely necessary to make the determination correct!) is missing from the description of the procedure? Compare with the first experiment described above.
- 15.5 Write down the reactions taking place in this procedure.
- 15.6 Calculate the amount of calcium in the sample.

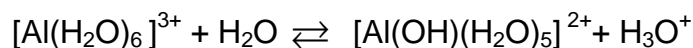
Similar principles are used in determination of silicic acid. To the neutralized colloidal solution of the sample, 0.5 g of KF was added, which was followed by introduction of HCl (10.00 cm³, $c = 0.0994 \text{ mol dm}^{-3}$) up to a definite excess. The resulting mixture was then

titrated with a standard solution of alkali in the presence of phenyl red (5.50 cm³ of NaOH solution with a concentration of 0.1000 mol dm⁻³ were spent).

- 15.7** What chemical reaction(s) is the determination based on? Write silicic acid as Si(OH)₄.
- 15.8** What indicator should be used when neutralizing the sample of silicic acid before the titration? The p*K*_a values of indicators: methyl red, 5.1; phenol red, 8.0; thymolphthalein, 9.9.
- 15.9** Calculate the amount of silicic acid in the sample solution.

SOLUTION OF PREPARATORY PROBLEM 15

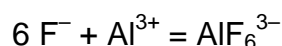
- 15.1** After the endpoint, the excessive Al³⁺ ions undergo hydrolysis, which makes the medium acidic, and the indicator turns red:



- 15.2** On heating, the hydrolysis equilibrium shifts rightwards.
- 15.3** Cryolite Na₃AlF₆ being formed upon the titration is only slightly soluble in water. Hence, NaCl was added to further decrease its solubility and shift the equilibrium of complex formation rightwards.
- 15.4** Neutralization of the sample solution before titration is missing. This operation is mandatory if an acid–base indicator is used to observe the endpoint and the sample is suspected to contain acids. Heating makes the endpoint sharper but is not as critical.
- 15.5** In this case a reverse titration was applied. Fluoride precipitates calcium:



and the excess of fluoride is titrated with AlCl₃:



15.6 10.25 cm³ of 0.1000 mol dm⁻³ AlCl₃ gives 1.025 mmol of Al³⁺, corresponding to 6.15 mmol of F⁻. The initial amount of NaF was 0.500 g, or 11.91 mmol, i.e. 5.76 mmol of F⁻ was spent for the precipitation of calcium. The amount of calcium is 2.88 · 10⁻³ mol.



As can be seen from the equation, HCl is spent in this process, and its excess is titrated with NaOH in the presence of an acid-base indicator. (To be more precise, the excess of HCl reacts with KF yielding a weak acid HF, which is then titrated with NaOH.)

15.8 The solution of free silicic acid (a weak acid with p*K*_a of about 10) will be slightly acidic; hence, the indicator used in the neutralization of the sample should change its color in a weakly acidic medium (methyl red, p*K*_a ≈ 5). In weakly alkaline media (color change range of two other indicators), a considerable part of the silicic acid will be present in the form of a silicate ion, the buffer solution of which will consume a certain amount of the reacting HCl.

The amount of NaOH and the excess of HCl are the same and equal to 0.550 mmol. Hence, the amount of HCl spent for the reaction with silicic acid is 0.994 – 0.550 = 0.444 mmol, and the amount of silicic acid is 0.111 mmol.

THEORETICAL 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 and α -diols undergo similar type of oxidation (Malaprade reaction). However, carboxylic, ester and aldehyde groups are not oxidized under these conditions.

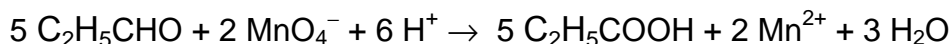
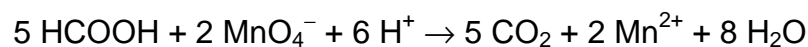
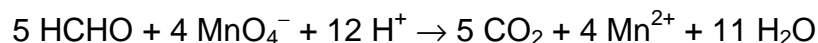
- 16.1** Provide the structures of organic products of the reaction of periodate with glycerol and butane-1,2-diol (mixture **A**).
- 16.2** A weighed amount of mixture **A** ($m_A = 1.64$ g) was introduced into the reaction with an excess of periodate, and the formed aldehyde groups were titrated with 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**.
- 16.3** A weighed amount of an individual compound **B** containing an amino group ($m_B = 105.0$ 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 $6.0 \cdot 10^{-3}$ 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.

SOLUTION OF PREPARATORY PROBLEM 16

16.1 With glycerol: $HCOOH + 2 HCHO$, with butane-1,2-diol: $C_2H_5CHO + HCHO$

16.2 $HCHO - 2 e^- \rightarrow HCOOH$; $HCOOH - 2 e^- \rightarrow CO_2$;
 $C_2H_5CHO - 2 e^- \rightarrow C_2H_5COOH$; $Mn^{+7} + 5 e^- \rightarrow Mn^{2+}$;

The complete reactions are:



The total mass of the mixture: $m_A = n_{\text{gly}}M_{\text{gly}} + n_{\text{but}}M_{\text{but}}$.

The amount of substance of $1/5 \text{KMnO}_4$ spent for the oxidation of aldehyde groups:

$n_{\text{ald}} = 4 \times 2 n_{\text{gly}}$ (2 mol of CH_2O from glycerol, $4 e^-$ each) + $2 n_{\text{gly}}$ (HCOOH from glycerol, $2 e^-$) + $2 n_{\text{but}}$ ($\text{C}_2\text{H}_5\text{CHO}$ from butylene glycol, $2 e^-$) + $4 n_{\text{but}}$ (1 mol of CH_2O from butylene glycol, $4 e^-$) = $10 n_{\text{gly}} + 6 n_{\text{but}}$,

Solving these two simultaneous equations (with $M_{\text{gly}} = 92$ and $M_{\text{but}} = 91$) one gets:

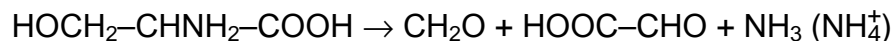
$n_{\text{but}} = 0.010 \text{ mol}$, $n_{\text{gly}} = 0.0079 \text{ mol}$.

- 16.3** The carboxylic group could either exist in the original compound **B** (a) or be formed during the oxidation. In the latter case, oxygen in **B** could be present in OH- and keto-groups (b) or only in OH-groups (c).

a) Let us suppose a minimum amount of oxygen-containing groups in **B**: 0.001 mol of $-\text{COOH}$ (45 mg) and two hydroxyl groups ($\equiv\text{C}-\text{OH}$ 29 g mol^{-1} $0.002 \text{ mol} = 58 \text{ mg}$); then, 0.001 mol of nitrogen should be also present (14 mg); this gives the total mass of 117 mg, which is even higher than the mass of **B** (105 mg). Therefore, a part of oxygen originates from the oxidant or water as a result of the substitution of amine nitrogen atom (which has transformed into the ammonium ion) with oxygen (so, amino groups in Malaprade reaction behave as hydroxyl ones). In case **B** contains one oxygen atom less, 1 mmol of $\equiv\text{C}-\text{OH}$ groups (29 mg) + 1 mmol of CHNH_2 (29 mg) + 1 mmol of COOH (45 mg) = 103 mg. To attain the required 105 mg, the following groups can be suggested: CHOH (30 mg), CH_2NH_2 (30 mg) and COOH (45 mg). Since 6.0 mmol equivalents of MnO_4^- were used, these could be spent for the titration of either 3.0 mmol $\text{HCOOH} / \text{RCHO}$ ($2 e^-$ each), or [1 mmol of formaldehyde ($4e^-$) + 1 mmol of $\text{HCOOH} / \text{RCHO}$ ($2e^-$)]. Only the second variant is consistent with the suggested composition of **B**. Thus, the formula of **B** is $\text{C}_3\text{H}_7\text{NO}_3$ (2-amino-3-hydroxypropionic acid or 3-amino-2-hydroxypropionic acid).

(b, c). These cases describe the situation when **B** is originally lacking the carboxylic group. If so, the molecular weight of 105 corresponds to compounds containing 1 oxygen atom less and 1 extra carbon atom ($C_4H_{11}NO_2$). If **B** contains only hydroxyl groups (case b), then it is a butane derivative containing 1 amino and 2 hydroxyl groups. If the butane moiety is unbranched and all three groups (two OH and an NH_2) are vicinal, then HCHO, HCOOH and CH_3CHO are formed upon oxidation with periodate. The amount of $KMnO_4$ spent for the titration would be $4 + 2 + 2 = 8$ mmol equivalents of $KMnO_4$, which is in contradiction with the available data. If OH and NH_2 groups are not vicinal, formaldehyde and an aldehyde are formed, but there would be no carboxylic acid produced. In case of isobutane derivatives, for instance, $HOCH_2-C(CH_3)NH_2-CH_2OH$, acetic acid and 2 moles of HCHO are formed requiring 8 equivalents of permanganate. $HC(CH_2OH)_2CH_2NH_2$ is not oxidized with periodate. If **B** contains a $C=O$ group (case c), its formula is $C_4H_9NO_2$ (molecular weight of 103), which is not consistent with the problem conditions. Consequently, only two compounds are left in consideration: 2-amino-3-hydroxypropionic acid (serine) and 2-hydroxy-3-aminopropionic acid.

Scheme of the **B** oxidation with periodate:



THEORETICAL PROBLEM 17

Analysis of Chrome Green

Chrome Green pigment is obtained by mixing lead(II) chromate and iron(II) hexacyanoferrate(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.

17.1 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 $\text{Na}_2\text{S}_2\text{O}_3$ solution in the presence of starch.

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

$\text{Na}_2\text{S}_2\text{O}_3$ solution should be standardized before using it as the titrant. The standardization is carried out against a standard $\text{K}_2\text{Cr}_2\text{O}_7$ solution in the same way as described above for the determination of chromate. If the acidity of the solution significantly exceeds 0.4 mol dm^{-3} , the reaction between dichromate and iodide induces the oxidation of iodide with atmospheric oxygen.

17.3 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 cm^3 out of the total volume of 50.0 cm^3) was used for the iodometric determination of chromate following the procedure described above (5.01 cm^3 of $\text{Na}_2\text{S}_2\text{O}_3$ solution ($c = 0.0485 \text{ mol dm}^{-3}$) were spent).

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

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

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

Another aliquot of the filtered solution (10.00 cm³ out of the total volume of 50.0 cm³) were mixed with 10.00 cm³ of K₄Fe(CN)₆ solution ($c = 0.0300 \text{ mol dm}^{-3}$) acidified with H₂SO₄ to obtain $[\text{H}^+] \cong 1 \text{ mol dm}^{-3}$ and titrated with KMnO₄ solution ($c = 0.00500 \text{ mol dm}^{-3}$) (2.85 cm³ were spent).

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

17.7 Calculate the amount of Turnbull's Blue in the sample (mg Fe₃[Fe(CN)₆]₂).

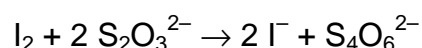
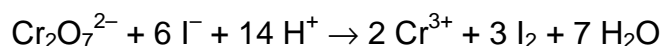
SOLUTION OF PREPARATORY PROBLEM 17

17.1 $\text{PbCrO}_4 + \text{Na}_2\text{CO}_3 \rightarrow \text{Na}_2\text{Pb(OH)}_4 + \text{Na}_2\text{CrO}_4 + \text{NaHCO}_3$



Fe(III) hydroxide is left on the filter.

17.2 Direct oxidation of thiosulfate with dichromate is not stoichiometric. The reactions normally used are:



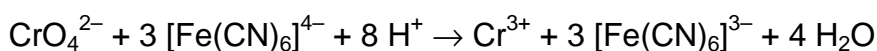
17.3 If reaction B is induced by reaction A, it implies that reaction A produces some intermediates active with the components of reaction B. In our case, the reduction of Cr(VI) occurs via the formation of intermediate oxidation states of chromium, predominantly Cr(V) species. (At the same time, the oxidation of I⁻ to I⁰ may not require any iodine-containing intermediates.) A reasonable reaction scheme is as follows:



As a result of oxygen involvement, a higher amount of free iodine is obtained, which results in a greater amount of Na₂S₂O₃ titrant spent and lower apparent concentration determined.

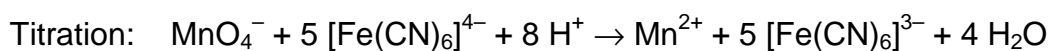
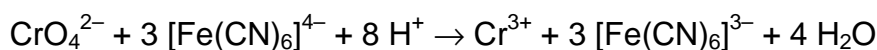
17.4 The amount of chromium is found as follows: $3 n_{\text{Cr}} = n_{\text{thios}} = 0.0485 \text{ mol dm}^{-3} \times 5.01 \text{ cm}^3 = 0.2430 \text{ mmol}$ ($n_{\text{Cr}} = 0.0810 \text{ mmol}$). This corresponds to 26.2 mg of PbCrO_4 ($M = 323.2 \text{ g mol}^{-1}$) in the aliquot, or 262 mg totally.

17.5 The side reaction



produces an amount of $[\text{Fe}(\text{CN})_6]^{3-}$ equivalent to CrO_4^{2-} reacted. At the titration stage that hexacyanoferrate(III) would also liberate free iodine; hence, the side process can be neglected.

17.6 Acidification of the sample:



17.7 On acidification of the 2nd aliquot, chromium is reduced by $[\text{Fe}(\text{CN})_6]^{4-}$ (see 17.5). Then permanganate is spent for the oxidation of $[\text{Fe}(\text{CN})_6]^{4-}$, namely, the amount of $[\text{Fe}(\text{CN})_6]^{4-}$ added plus the amount contained initially in the sample less the amount spent for the reduction of Cr(VI):

$$5 n(\text{MnO}_4^-) = n(\text{Fe added}) + n(\text{Fe from sample}) - 3 n(\text{Cr}).$$

From this equation one can find $n(\text{Fe from sample})$:

$$\begin{aligned} n(\text{Fe from sample}) &= 5 n(\text{MnO}_4^-) - n(\text{Fe added}) + 3 n(\text{Cr}) \\ &= 5 \times 0.00500 \times 2.85 - 10 \times 0.0300 + 0.2430 = 0.07125 - 0.3000 + 0.2430 = \\ &= 0.0155 \text{ mmol}. \end{aligned}$$

This corresponds to 4.44 mg of $\text{Fe}_{4/3}[\text{Fe}(\text{CN})_6]$ ($M = 286.3 \text{ g mol}^{-1}$) in the aliquot, or 22.2 mg totally.

THEORETICAL 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.

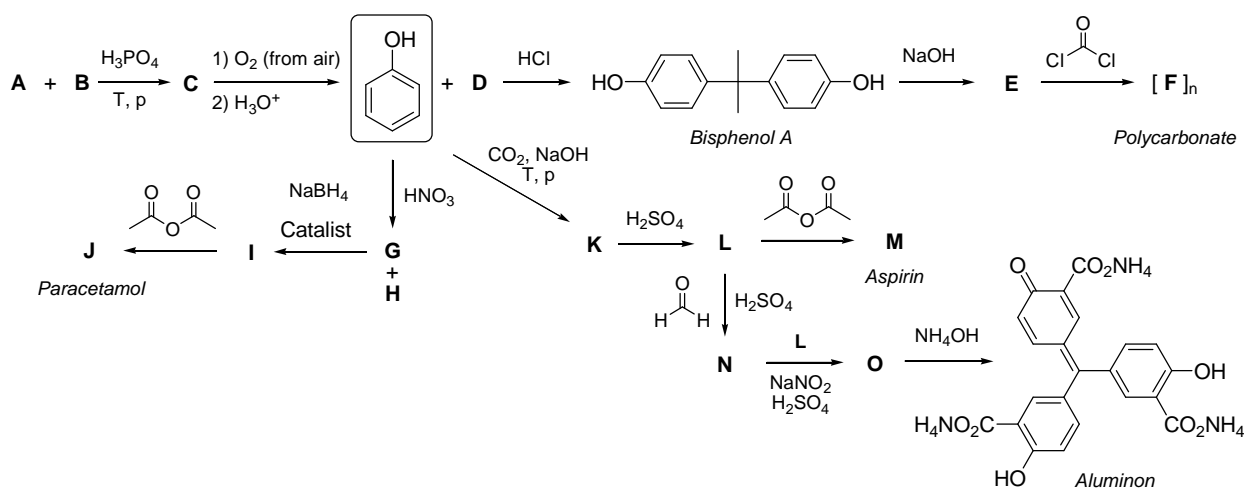
High potential of phenol in the synthesis of polymers, drugs, and dyes can be illustrated by the hereunder examples.

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 CO₂ 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 NaNO₂ and sulfuric acid yields **O**, which finally gives *Aluminon* upon treatment with ammonia.



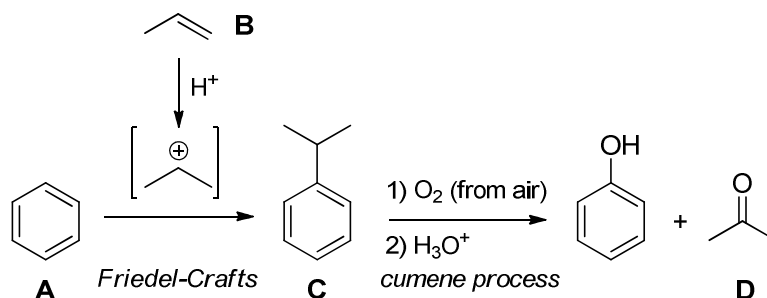
18.1 Write down the structural formulae of **A – E** and **G – O**.

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

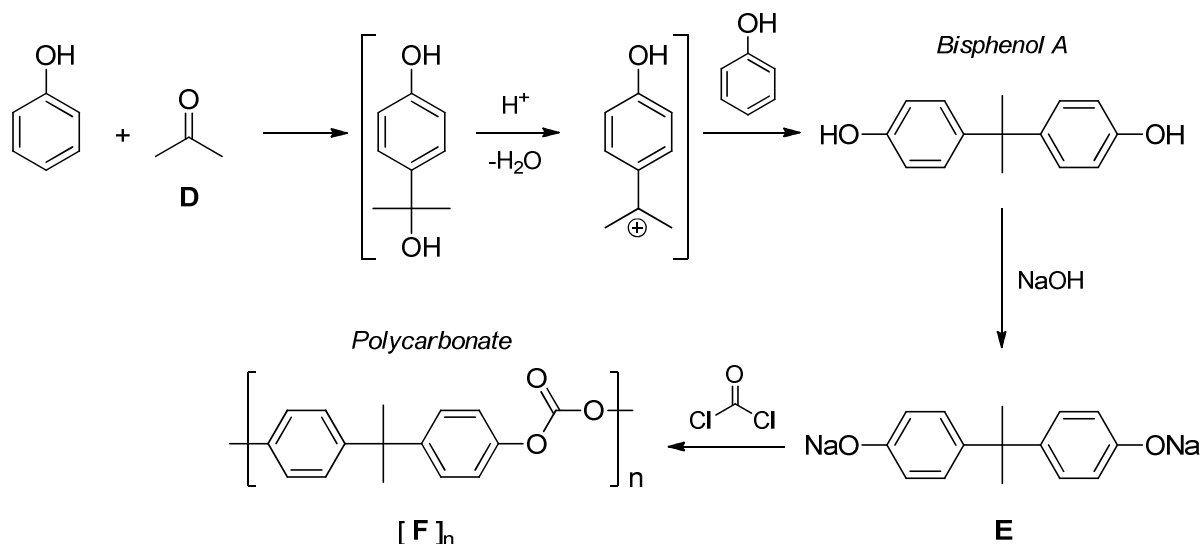
SOLUTION OF PREPARATORY PROBLEM 18

18.1 Structures of benzene **A** and propene **B** are commonly known.

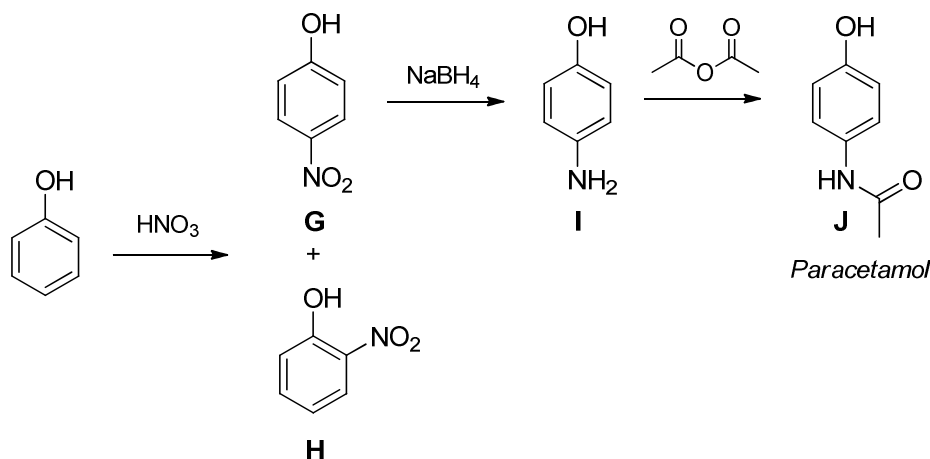
The interaction between **A** and **B** under acidic condition proceeds as Friedel-Crafts alkylation of the aromatic ring with the thermodynamically more stable secondary propyl carbocation as an electrophile. Being a product of the interaction of equal amounts of **A** and **B**, **C** turns out to be isopropylbenzene, i.e. cumene. Oxidation of **C** with subsequent acidification leads to phenol and acetone **D**. This classical industrial procedure is known as *cumene process*.



The structure of **D** can also be easily determined from that of *bisphenol A*, which is formed as a result of two stepwise Friedel-Crafts alkylations of phenol. Treatment of *bisphenol A* with NaOH leads to sodium bis-phenolate **E**, which gives *polycarbonate* with the monomeric unit **F** as a result of the reaction with phosgene.

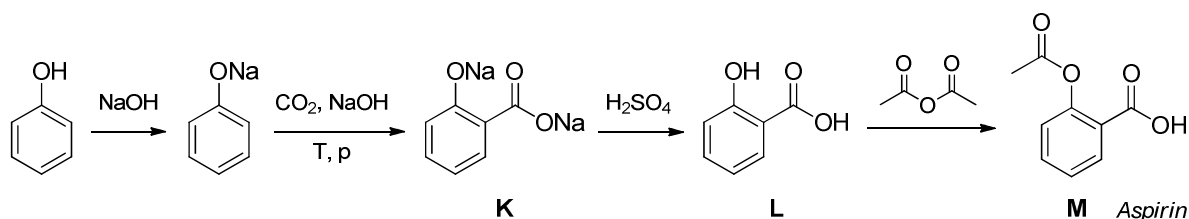


The reaction of phenol with diluted nitric acid proceeds as a mononitration resulting in isomeric nitrophenols **G** and **H**. Due to the activation effect of OH-group in phenol, electrophilic substitution can occur in *ortho*- and *para*-positions of phenol. **G** is *para*-nitrophenol (two planes of symmetry), whereas **H** is *ortho*-nitrophenol (only one plane of symmetry). Further reduction of NO_2 -group in *para*-nitrophenol **G** results in *para*-aminophenol **I**. Due to its higher nucleophilicity, NH_2 -group (rather than OH-group) in **I** is acetylated with acetic anhydride giving *paracetamol* **J**.

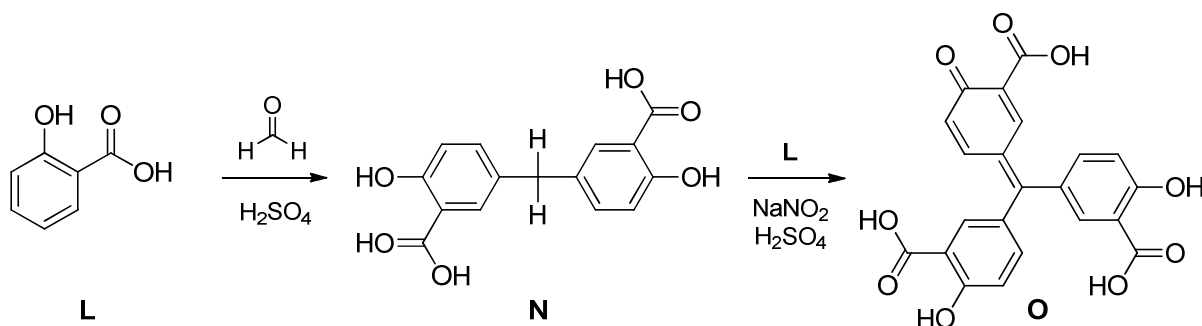


The reaction of phenol with CO_2 in the presence of NaOH proceeds through intermediate formation of sodium phenolate, which interacts with CO_2 under heating and high pressure (Kolbe-Schmitt reaction) to give disodium salicylate **K**.

Acidification of **K** with two equivalents of an acid results in salicylic acid **L**, which provides *aspirin* **M** when acetylated with acetic anhydride.



Aluminon synthesis is based on the same approach as previously considered for *bisphenol A*. The reaction of salicylic acid **L** with formaldehyde under acidic conditions affords **N**, which is an analogue of *bisphenol A*. Addition of another equivalent of salicylic acid **L** under oxidative conditions ($\text{NaNO}_2/\text{H}_2\text{SO}_4$) gives the tri-acid **O**, which is a direct precursor of *Aluminon*. Thus, the structure of **O** can be derived from that of *Aluminon*.

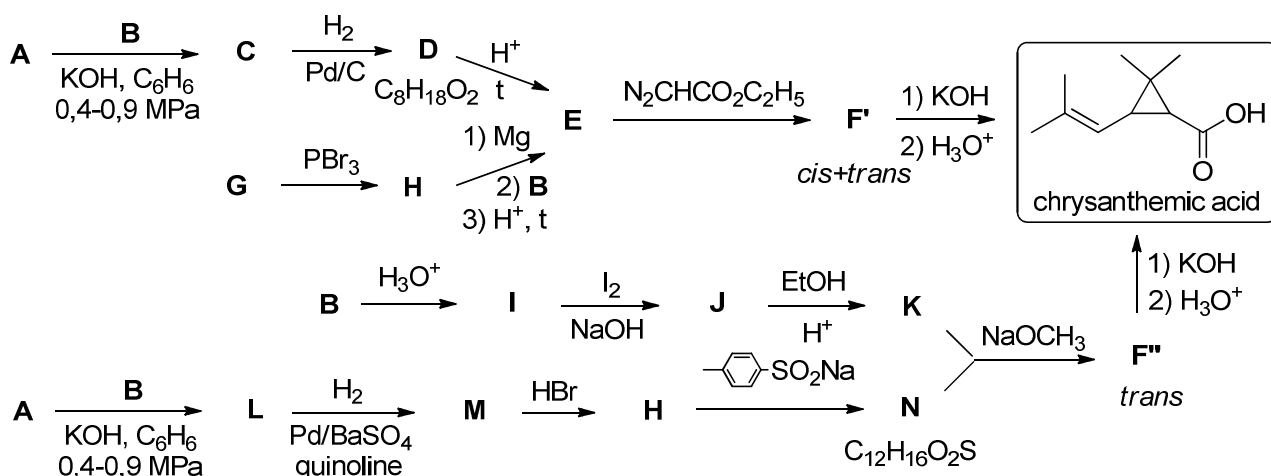


THEORETICAL PROBLEM 19

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).

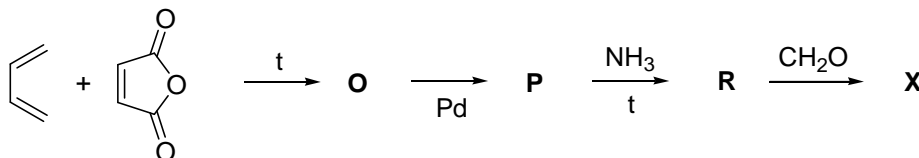


19.1 Write down the structural formulae of all compounds given in this scheme. Note that **A** is a gaseous hydrocarbon with the density lower than that of air, **G** is a natural alcohol, **F'** is a mixture of isomers, whereas **F''** is formed only in trans-form.

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

19.2 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.



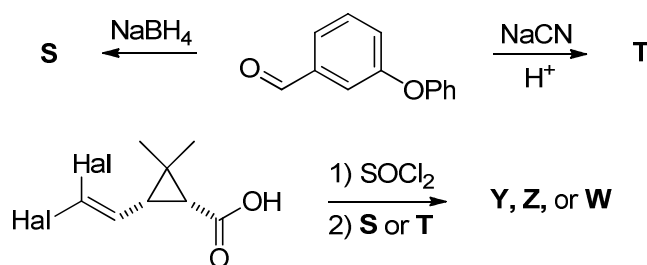
19.3 Write down the structural formulae of **O-R**, and **X**. Note that the transformation of **O** into **P** is an isomerization with retention of the carbocyclic skeleton leading to the most stable isomer.

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

19.4 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 $\text{CH}=\text{C}(\text{CH}_3)_2$ fragment in chrysanthemic acid by the $\text{CH}=\text{CHal}_2$ 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.

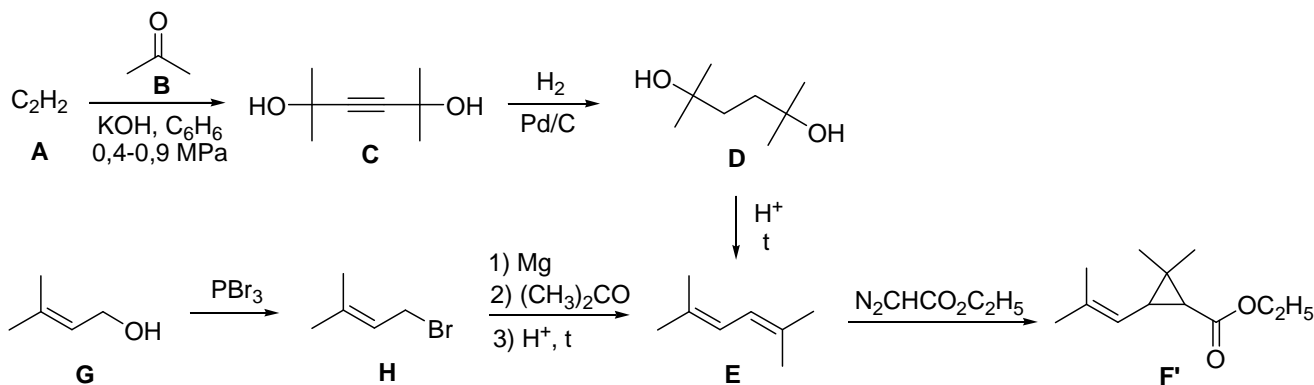


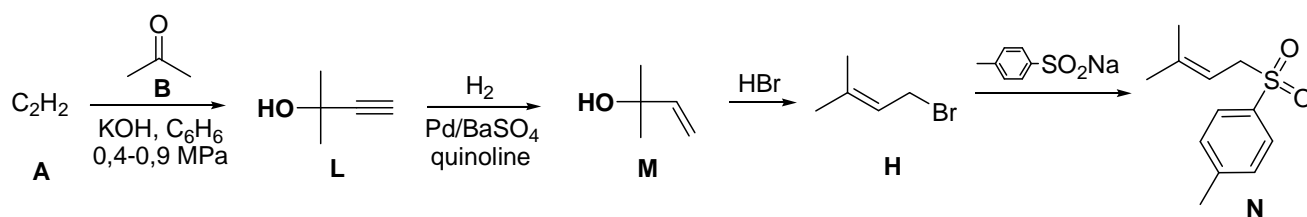
19.5 Write down the structural formulae of **S**, **T**, **W**, **Y**, **Z**. Note that the halide content in **W**, **Y**, **Z** is 31.6, 18.1, and 17.0 %, respectively.

SOLUTION OF PREPARATORY PROBLEM 19

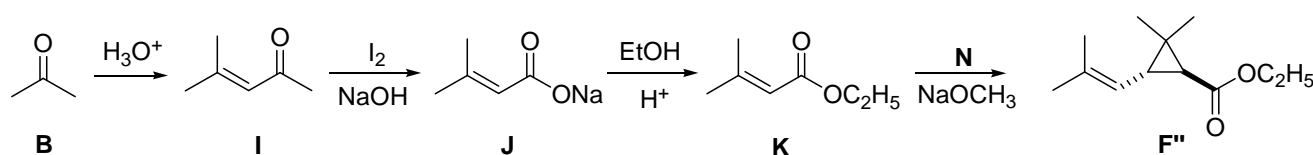
19.1 Chrysanthemic acid is formed as a result of hydrolysis of its ethyl ester, **F**, which, in turn, is obtained by cyclopropanation of **E** with diazoacetic ester. Therefore, **E** is 2,5-dimethylhex-2,4-diene with molecular formula C_8H_{14} . This conclusion is supported by the molecular formula of **D**. Evidently, transformation of **D** to **E** is elimination of two water molecules.

Eight carbon atoms of **D** originate from **A** and **B**. The other reaction between these compounds affords **L** containing 5 carbon atoms (**N** is formed from **H** and $C_7H_7O_2SNa$; the number of carbon atoms in **H** and **L** is the same). The provided information strongly suggests that **A** is acetylene (C_2H_2). Hence, **A** is composed of 2, and **B** should be composed of 3 carbon atoms. Reaction between **A** and **B** was disclosed by Favorskii in 1905 as that between acetylenes and carbonyl compounds. It means that **B** is either propionic aldehyde (C_2H_5CHO) or acetone (CH_3COCH_3). Accounting for the structure of **E**, **B** is acetone. **E** is also formed through the Grignard reaction of acetone with the corresponding $RMgBr$ followed by elimination of water. The structure of **H** can be unambiguously deduced from that of **E** – it is prenyl bromide. So, the natural alcohol is prenil (3-methylbut-2-en-1-ol). **L** is formed from **A** and **B** under the same reaction conditions but when **A** to **B** ratio is of 1:1. Therefore, **L** is 2-methylbut-3-yn-2-ol. Its hydrogenation in the presence of Lindlar catalyst leads to the corresponding alkene **M**. Subsequent reaction with HBr affords prenyl bromide **H** via nucleophilic substitution with double bond migration. The reaction of **H** with sodium 4-toluenesulfinate results in the corresponding sulfone **N**.

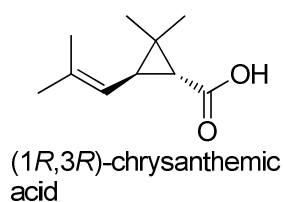




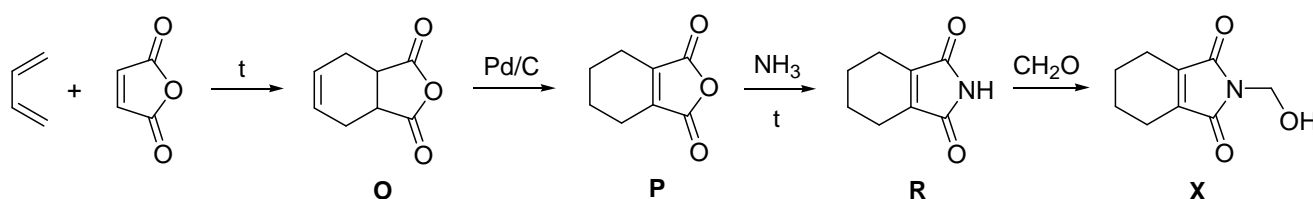
Lastly, acid-catalyzed self-condensation of acetone yields 4-methylpent-3-en-2-one (mesityl oxide, **I**). Iodoformic reaction of **I** produces the corresponding acid **J** which is further transformed into ethyl ester **K**. The reaction of **K** with deprotonated sulfone **N** results in chrysanthemic acid.



19.2

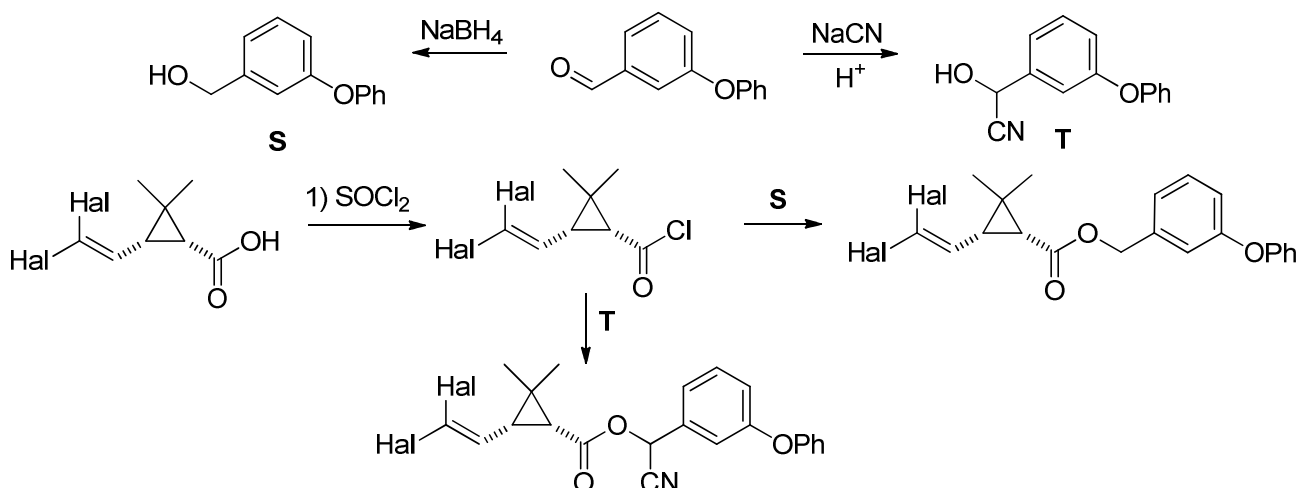


19.3 The first step is the Diels-Alder reaction. Compound **P** with tetrasubstituted double bond is the most stable isomer of **O** with the same carbocyclic skeleton. Heating of **P** with ammonia leads to imide **R**, which further reacts with CH_2O giving the target alcohol **X**.



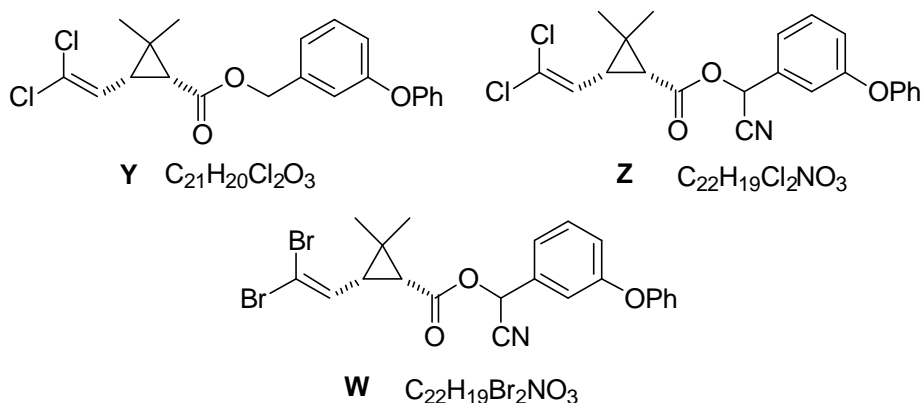
19.4 Amides and hydrazides do not easily form esters in reaction with alcohols. Oppositely, anhydrides are appropriate reagents for the ester synthesis. Moreover, re-esterification of methyl or ethyl esters with high-boiling alcohols is well-known. These reactions are efficient due to methanol (ethanol) removal from the reaction mixture via distillation (Le Chatelier's principle).

19.5 Reduction of 3-phenoxybenzaldehyde yields the corresponding benzyl alcohol **S**, while its reaction with NaCN produces cyanohydrin **T**. Reaction of **S** or **T** with 2,2-(dihalovinyl)-3,3-dimethylcyclopropane-1-carbonyl chloride affords the target pyrethroids.



Molecular formulae of the esters formed from alcohol **S** and **T** are $C_{21}H_{20}Hal_2O_3$ and $C_{21}H_{19}Hal_2NO_3$, respectively. Halide content in the esters is $2M_{Hal}/(2M_{Hal} + 320)$ and $2M_{Hal}/(2M_{Hal} + 345)$, respectively. Calculation of halide content in these compounds allows unambiguously deciding on the structures of the pyrethroids.

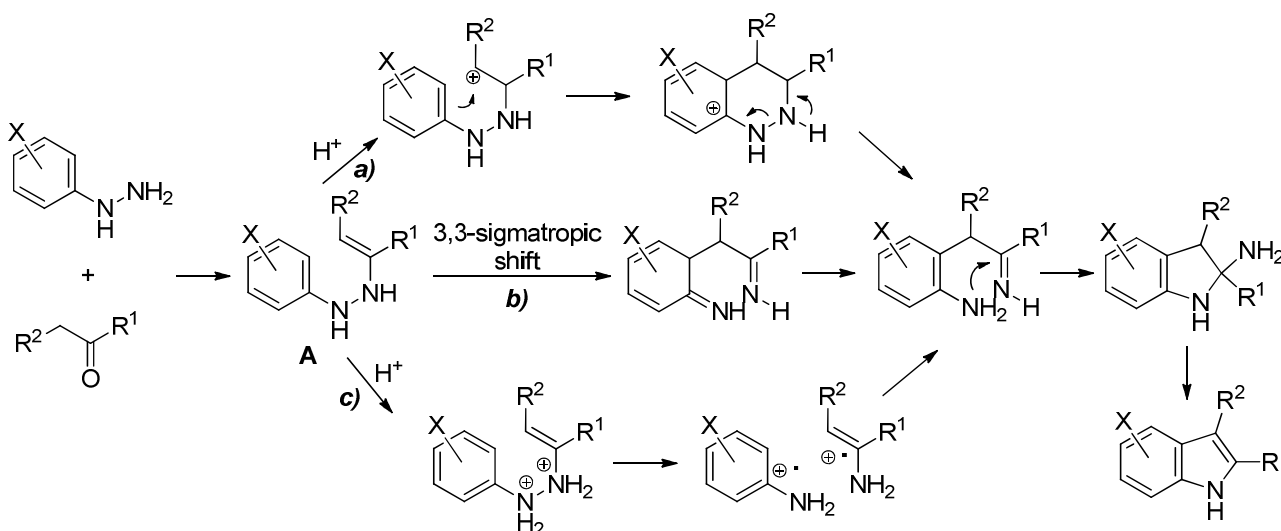
Content of Hal, %	Using exact atomic mass				Using approximate atomic mass			
	F	Cl	Br	I	F	Cl	Br	I
Ester of S	10.60	18.12	33.28	44.20	10.61	18.16	33.33	44.25
Ester of T	9.91	17.03	31.63	42.36	9.92	17.07	31.68	42.40



THEORETICAL 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.



20.1 Write down the mechanism of enhydrazone **A** formation.

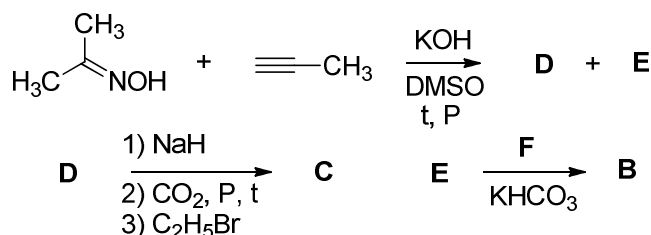
In 1970s, the Russian scientist I. Grandberg investigated a reaction of *N,N*-diarylhazines Ar¹Ar²NNH₂ with ketones and discovered that a mixture of two indoles in a ratio of ca. 1:1 is formed, the result being independent of the substituent nature (donor or acceptor) in the aryl groups. These experiments proved unambiguously the mechanism of the Fischer indole synthesis.

20.2 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.

20.3 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**.

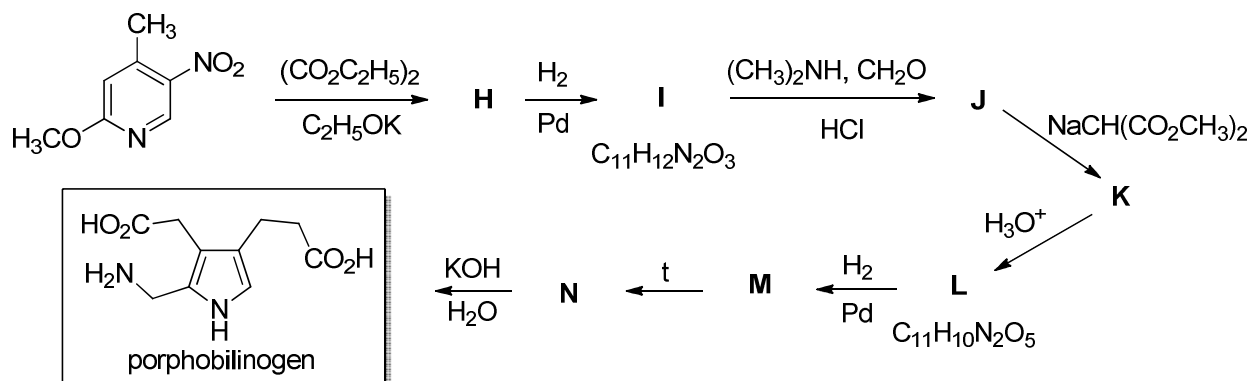


20.4 Write down the structural formulae of **D-F**. Note that the carbon content in **F** is 28.7%.

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.

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

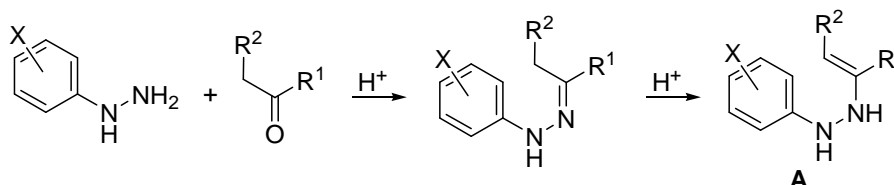
Pyrrole ring is a key moiety of many bioactive natural compounds including porphobilinogen, an intermediate in biosynthesis of heme and chlorophyll. This compound was synthesized in laboratory according to the hereunder scheme.



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

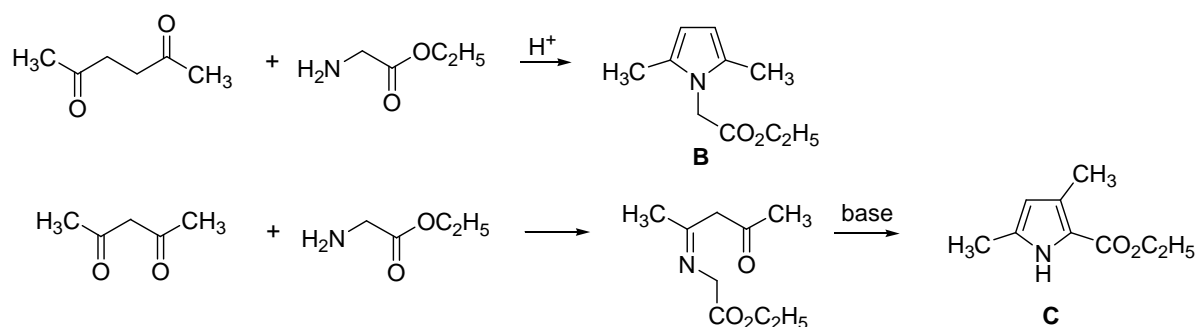
SOLUTION OF PREPARATORY PROBLEM 20

20.1 Interaction of ketone with arylhydrazine affords hydrazone, which isomerizes into enhydrazone under acidic conditions.



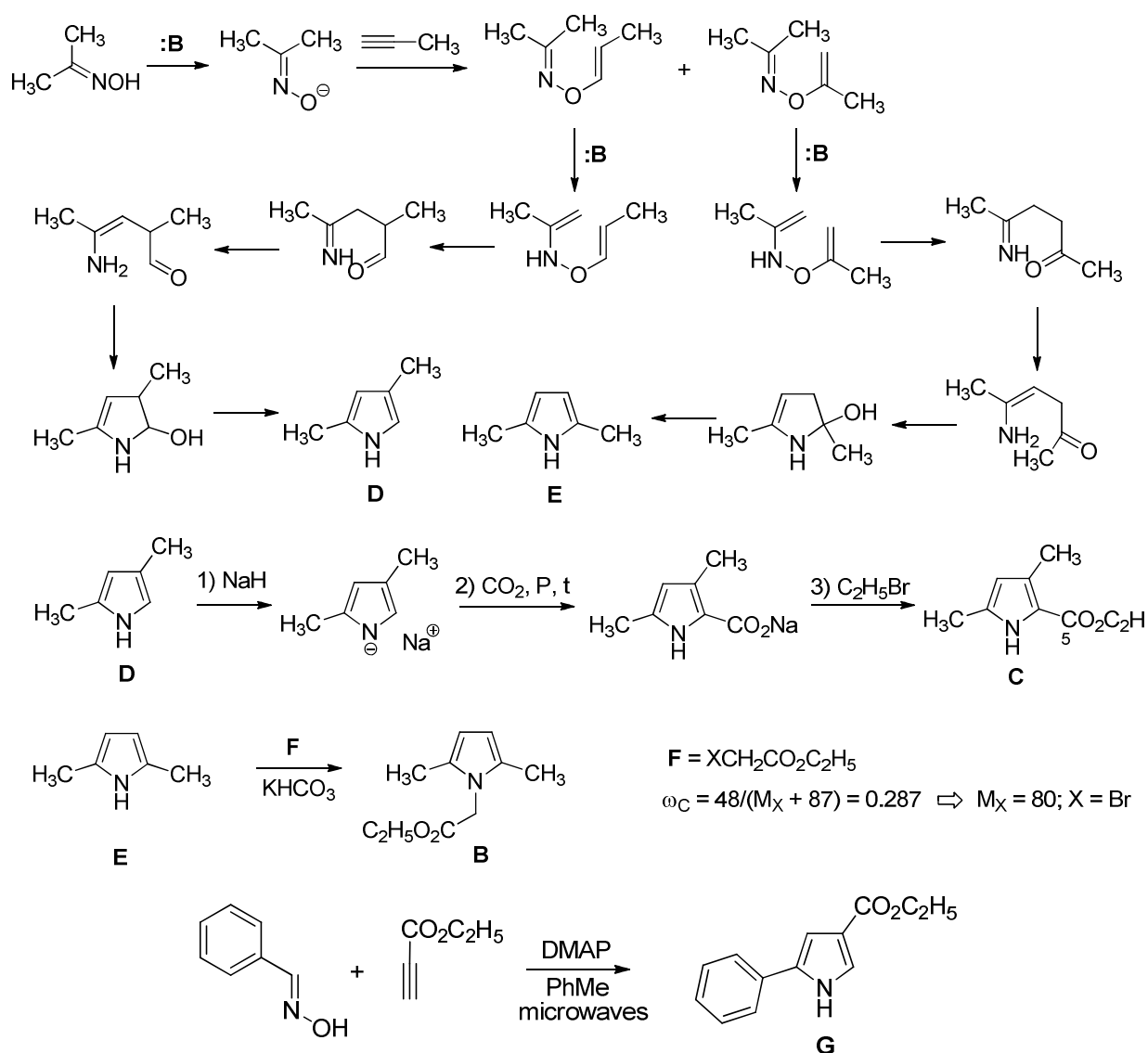
20.2 Mechanism **a** includes the electrophilic attack of aminoalkyl cation at the aromatic moiety. This attack is very susceptible to electron properties of the aryl group (attack on the electron-enriched aryl ring is much more efficient than that on the electron-depleted arene). The same is expected for mechanism **c**. Only the sigmatropic shift has no significant dependence on substituents in both arenes. Therefore, I. Grandberg proved that the Fischer indole synthesis proceeds via mechanism **b**.

20.3 Reactions are started by interaction of amine with the carbonyl group furnishing imine. To complete pyrrole moiety formation, monoimine of hexane-2,5-dione should isomerize into the enamine followed by an attack of the amine group on the second C=O group. Formally, imine of pentane-2,4-dione can form the pyrrole ring in two ways. First, it is the interaction of the nitrogen atom with the methyl group. However, the methyl group itself is unreactive towards nucleophiles. Keto-enol equilibrium with involvement of the methyl group in this compound is less probable than that with CH₂-fragment. Even if the equilibrium was true, enol is a nucleophile and cannot react with nucleophilic nitrogen atom. Therefore, the second possibility should be considered, namely, the reaction of the second carbonyl with CH₂ bound to N atom. This reaction is quite probable as CH₂-group is also connected with the electron-withdrawing ester group and can be deprotonated by a base as shown below.



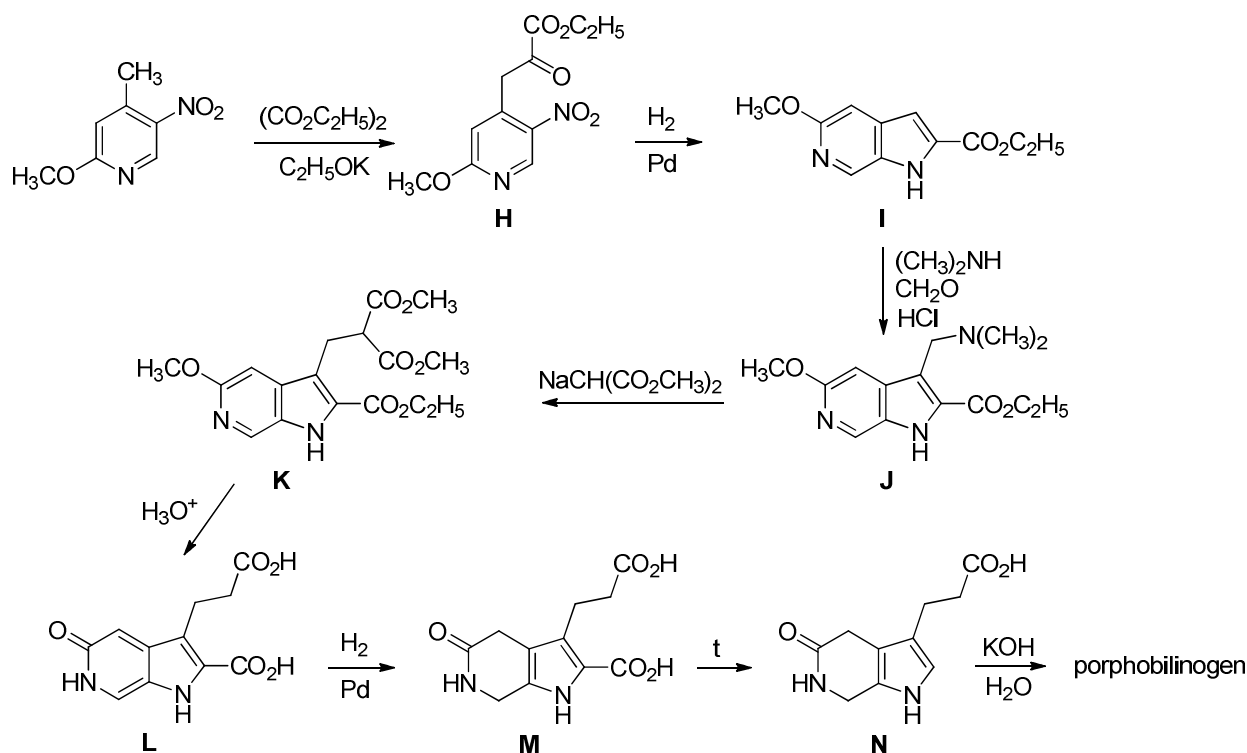
20.4, 20.5

Two products are formed in the reaction of propyne, and only one product in the case of the alkyne bearing an electron-withdrawing ester group. This allows supposing a nucleophilic attack of a certain intermediate on the alkyne moiety. A base generates a nucleophilic agent from acetone oxime. Again, two ways of deprotonation are possible: *O*-deprotonation and *C*-deprotonation. However, oxime enolate, if formed, should add to alkyne with the formation of hex-4-en-2-one oxime. There is no possibility for the transformation of this oxime into pyrrole ring. The alternative possibility is *O*-deprotonation and nucleophilic addition of the oximate ion to alkyne furnishing *O*-alkenyl acetone oxime. Formation of the C-C bond between the methyl group of acetone and the β -carbon atom of the alkenyl group is needed to complete the pyrrole ring synthesis. At the first glance, such transformation is impossible. However, this system is very similar to the *N*-aryl-*N*'-alkenyl moiety which undergoes the 3,3-sigmatropic rearrangement in the Fischer indole synthesis. Indeed, isomerization of *O*-alkenyl acetone oxime into *O*-alkenyl-*N*-alkenyl derivative creates the fragment required for the 3,3-sigmatropic shift. So, formation of the pyrrole ring giving 2,4-dimethylpyrrole and 2,5-dimethylpyrrole is analogous to that of indole in the Fischer synthesis. The former compound is transformed into **C** via *N*-deprotonation followed by the Kolbe-Schmitt carboxylation and ester formation. **E** is *N*-alkylated with ethyl haloacetate. Halogen can be determined from the carbon content in the alkylation reagent.



20.6 Methyl group in the starting compound is very acidic due to activation by both *ortho*-nitro group and *para*-nitrogen atom of pyridine. So, it can be easily deprotonated to further react with diethyl oxalate providing the corresponding ketoester **H**. Reduction of the nitro group gives aniline. Condensation of the amino group with the appropriately located ketone moiety affords the 6-azaindole derivative **I** ($\text{C}_{11}\text{H}_{12}\text{N}_2\text{O}_3$). Aminomethylation of this indole furnishes the gramine derivative **J** which undergoes nucleophilic substitution with sodium dimethylmalonate producing **K**. Its hydrolysis results in a compound with the molecular formula of $\text{C}_{11}\text{H}_{10}\text{N}_2\text{O}_5$. It means that: a) hydrolysis of the malonate fragment is accompanied by decarboxylation; b) the ester moiety at the C2 position of the indole is hydrolyzed too. However, even if so, the molecular formula should be $\text{C}_{12}\text{H}_{14}\text{N}_2\text{O}_3$. The

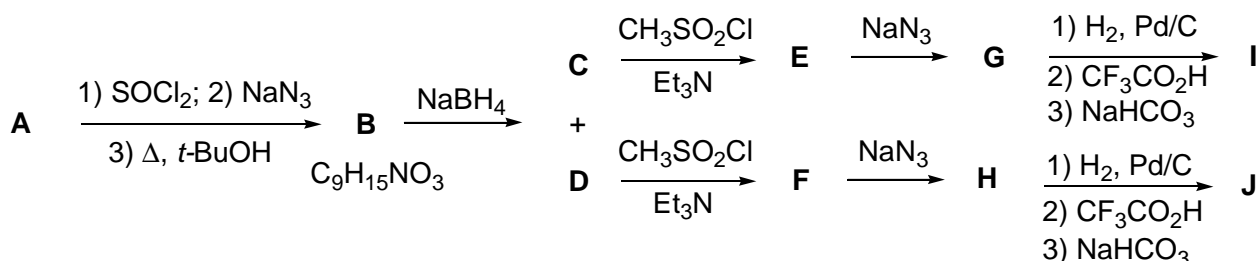
difference equals to CH_2 . Hydrolysis of OCH_3 -group in *ortho*-position to pyridine nitrogen is the only possibility. Indeed, hydrogenation of this pyrrolopyridone yields **M**. Its decarboxylation and hydrolysis of the amide function finally leads to porphobilinogen.



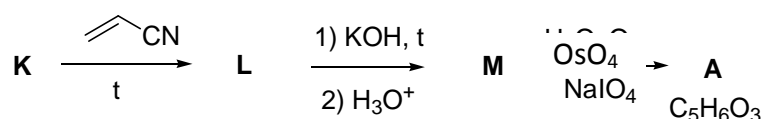
THEORETICAL PROBLEM 21

Cyclobutanes

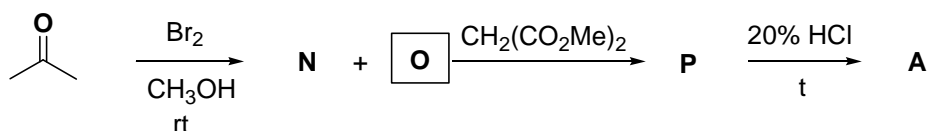
In 1894 Emil Fischer proposed the “lock and key” principle for interaction between a drug and its molecular target. The interaction is efficient only in case of substances having specific complementary geometry that fit exactly to the molecular target. According to this model, a potential drug should 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:



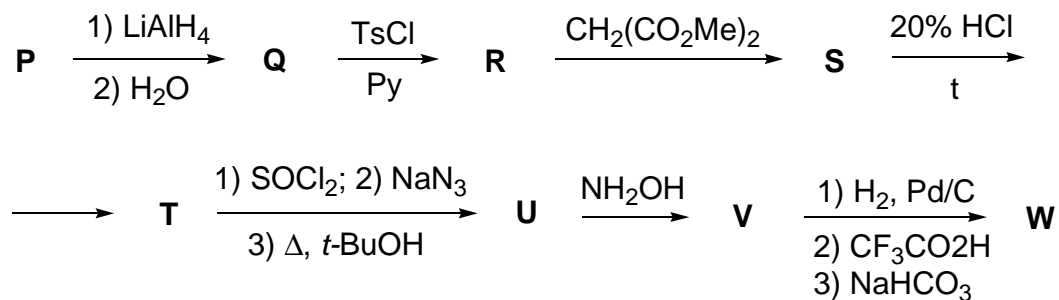
Another method for **A** synthesis is given below:



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

- C** and **D** are isomers; **J** has two planes of symmetry;
- hydrocarbon **K** has a single type of hydrogen atoms; $w_{\text{H}} = 10.0\%$;
- N** and **O** are isomers; $w_{\text{H}} = 3.8\%$; $w_{\text{C}} = 22.9\%$.

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

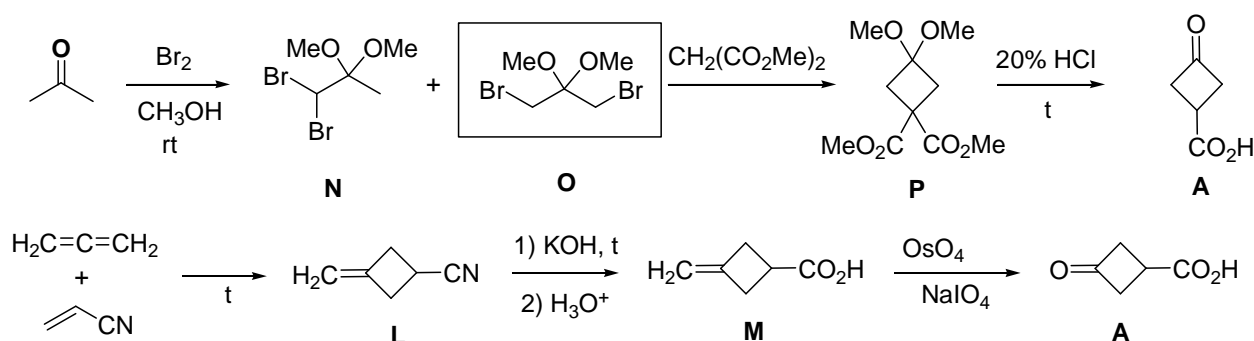


21.2 Write down the structural formulae of **Q** – **W**.

21.3 Can **W** be resolved into enantiomers?

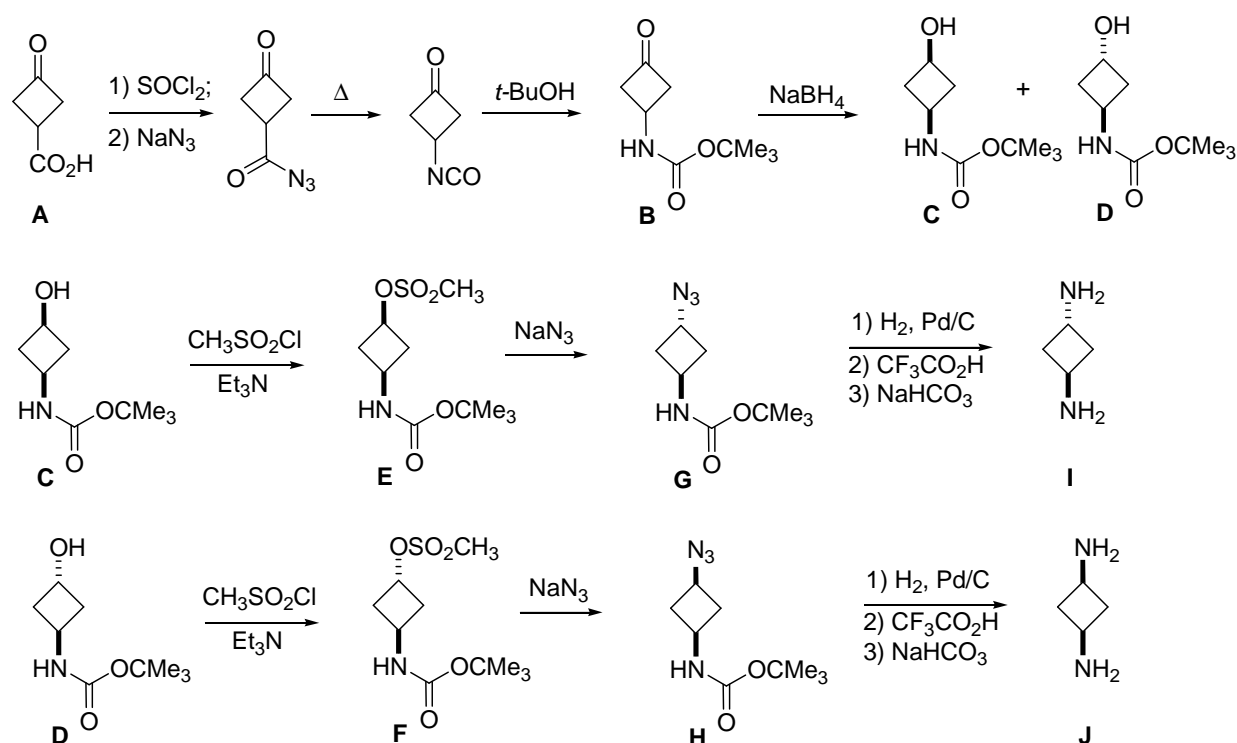
SOLUTION OF PREPARATORY PROBLEM 21

21.1 Hydrocarbon **K** consists of 90% C and 10% H. Its simplest formula is $(C_3H_4)_n$, and it has a single type of H atoms. So, it is allene, $H_2C=C=CH_2$. **A** has 5 carbon atoms. Therefore, allene reacted with $CH_2=CHCN$ in a ratio of 1:1 and lost one carbon atom during the following steps. Various products can be supposed for this reaction, however, it is known that allene is prone to undergo cycloaddition as 2π -component. Acrylonitrile undergoes cycloaddition as 2π -component too. So, the product should be a cyclobutane derivative, which is consistent with the next scheme. The C and H content in **N** and **O** provides for their molecular formula $(C_5H_{10}Br_2O_2)$. In this respect, two sub-processes should proceed: a) acetone is doubly brominated; b) ketone is transformed into ketal in the reaction with methanol catalyzed by HBr evolved in the bromination step. Two dibromoacetones (1,1- and 1,3-) can be formed. Reaction of the latter with dimethyl malonate affords the corresponding cyclobutane derivative. Its treatment with hydrochloric acid leads to the hydrolysis of ketal into ketone and esters into an acid. So, the product should be 3-oxocyclobutane-1,1-dicarboxylic acid. However, its formula is $C_6H_6O_5$. Therefore, hydrolysis is also accompanied by decarboxylation of the malonic acid moiety. So, **A** is 3-oxocyclobutanecarboxylic acid. Accounting for it, **L** is the product of [2+2] cycloaddition, *i.e.*, 1-cyano-3-methylenecyclobutane. Its hydrolysis followed by oxidation of C=C double bond produces **A**. Finally, the schemes for preparation of **A** are as follows:



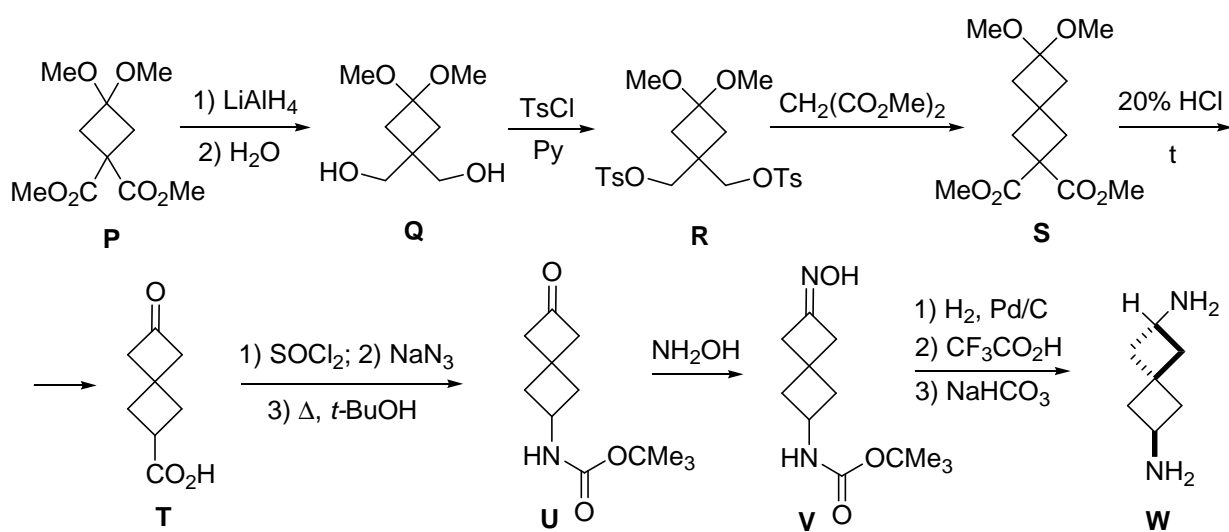
Reaction of **A** with $SOCl_2$ furnishes acyl chloride, which reacts with NaN_3 affording acyl azide. Heating of $RCON_3$ produces isocyanate $R-N=C=O$, which immediately reacts with *t*-BuOH giving rise to *N*-Boc-protected 3-aminocyclobutanone **B**.

Reduction of keto group with NaBH_4 leads to *cis*- and *trans*-isomers of the corresponding aminocyclobutanol. Further reaction with $\text{CH}_3\text{SO}_2\text{Cl}$ produces mesylates, which undergo $\text{S}_{\text{N}}2$ displacement with NaN_3 affording aminoazides. Reduction of azido group and deprotection of amine furnishes *cis*- and *trans*-isomers of 1,3-diaminocyclobutane. Therefore, **J** is *cis*-1,3-diaminocyclobutane (two planes of symmetry), and **I** is *trans*-isomer (one plane of symmetry). Similarly, **G** is *trans*-, and **H** is *cis*-isomer. As the $\text{S}_{\text{N}}2$ reaction proceeds with inversion of the configuration, compound **E** (leading to **G**) is *cis*-, and **F** is *trans*-isomer.



21.2, 21.3

Reduction of **P** with LiAlH_4 gives the corresponding diol **Q**, which is transformed into ditosylate **R**. Reaction of **R** with dimethyl malonate leads to formation of the second cyclobutane ring (**S**). Hydrolysis of **S** proceeds similarly to that of **P**, *i.e.*, it produces ketoacid **T**. Further transformations are also similar to those in the first scheme and produce spiro[3.3]heptane-2,6-diamine **W**. This compound has no plane or center of symmetry. It is chiral due to axial chirality (similarly to 1,3-disubstituted allenes), thus, it can be resolved into two enantiomers.



THEORETICAL 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.

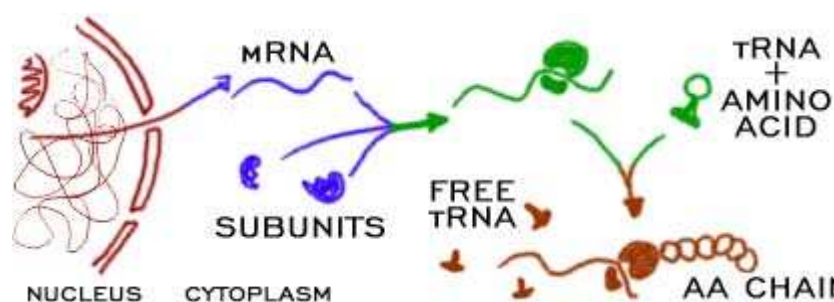


Figure 1. General scheme of protein translation in a living cell

(http://www.biology4kids.com/files/cell_ribos.html)

22.1 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?

22.2 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.

22.3 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.

- 22.4** 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.
- 22.5** Using the table of genetic code write down amino acid sequences for the oligopeptides:
- encoded by the hereunder mRNA
 - encoded by the hereunder mRNA with the first and the last C replaced by U
 - encoded by the hereunder mRNA with the first G replaced by C
 - encoded by the hereunder mRNA with the last but one G replaced by U

5'AUGGAUCACGCCAUCAAUGUUGUCGGUUGGAGUGUGGAUACGUUGGAU
GAUGGAACUGAAGCU3'.

- 22.6** 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).
- 22.7** 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 molar mass of an amino acid as 110 g mol^{-1} , 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).

- 22.8** 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.

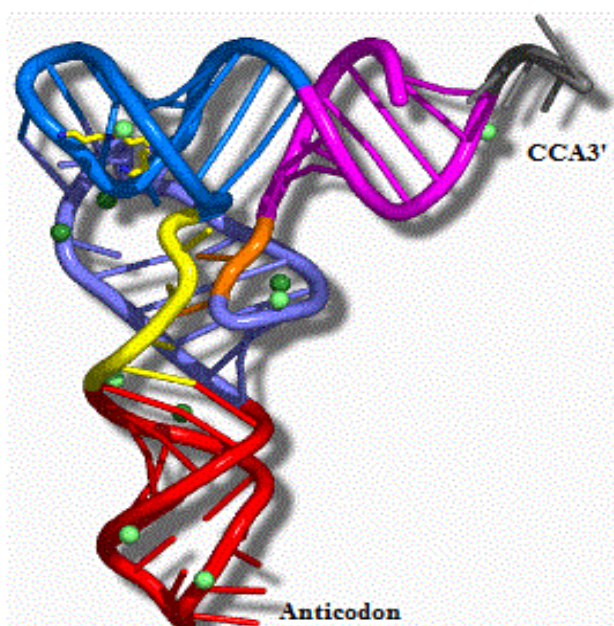


Fig. 2. The 3D structure of a tRNA

22.9 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.

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

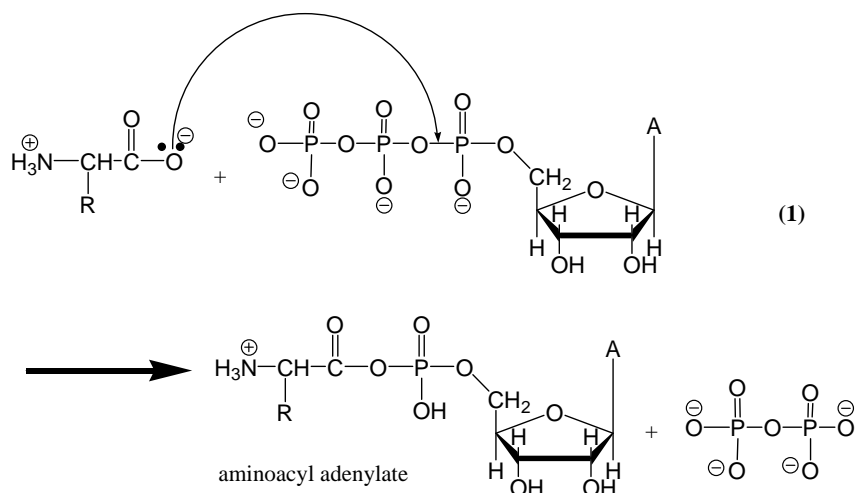
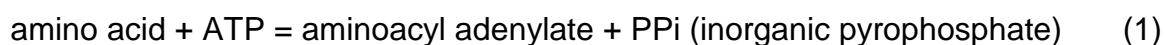
SOLUTION OF PREPARATORY PROBLEM 22

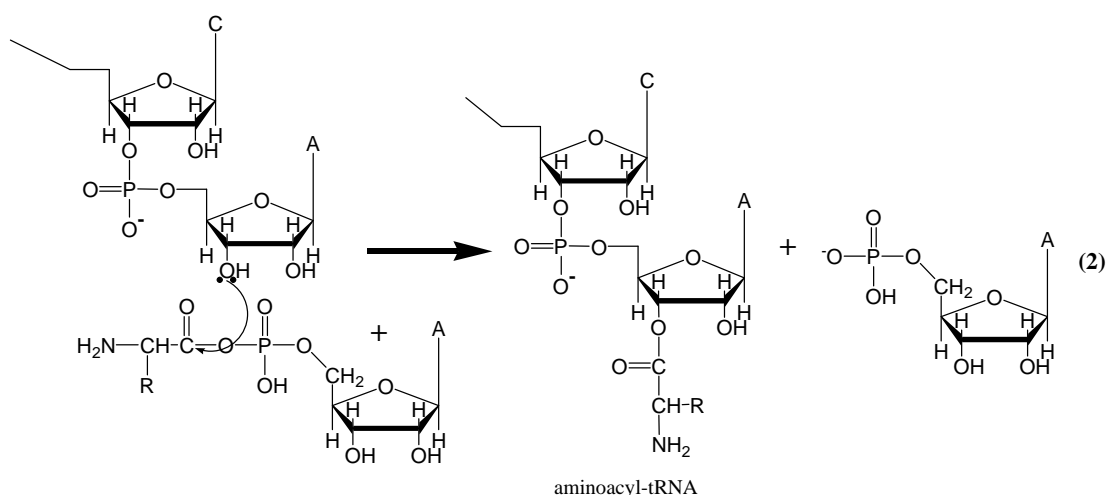
22.1 There are $4^3=64$ different three-nucleotide combinations of 4 nucleotides. Only 61 codons encode amino acids added to the growing polypeptide chain. 3 remaining combinations are STOP codons determining termination of the translation process.

22.2 No, because of redundancy of the genetic code: most amino acids are encoded by several codons.

22.3 Leucine is encoded by 6 different codons, thus it is delivered to a ribosome by 6 different tRNAs. Being encoded by only 1 codon, methionine is transported by a sole tRNA. In some organisms the latter codon is also responsible for the translation start, encoding the N-terminal amino acid N-formylmethionine. Still, methionine and N-formylmethionine are transported by different tRNAs.

22.4 The equations of consecutive reactions are:





Thus, the carboxylic group of the amino acid reacts with 3'-OH group of its tRNA.

- 22.5** a) Met-Asp-His-Ala-Ile-Asn-Val-Val-Gly-Trp-Ser-Val-Asp-Thr-Leu-Asp-Asp-Gly-Thr-Glu-Ala or fMet-Asp-His-Ala-Ile-Asn-Val-Val-Gly-Trp-Ser-Val-Asp-Thr-Leu-Asp-Asp-Gly-Thr-Glu-Ala, depending on the biosynthesizing species (Eukaryotes, Prokaryotes, or Archaea).
- b) The third amino acid is tyrosine, and the last one is valine. All the rest positions are the same.
- c) The N-terminal amino acid is leucine. All the rest positions are the same. It should be noted that the translation in bacteria would not start without the START codon.
- d) The last but one codon is changed into STOP codon, which will result in the oligopeptide shorter by 2 amino acid residues than that in i. 5a.

22.6 AUG-GAU/C-GUN-AAU/C-CAU/C-CCN-GAA/G-UAU/C-GGN-AAA/G

22.7 The protein consists of $51000/110 \approx 464$ amino acid residues.

Hence, it is encoded by the mRNA containing $464 \cdot 3 + 3 = 1395$ nucleotide residues including the STOP codon.

The length of mRNA is $1395 \cdot 0.34 = 474.3 \approx 474$ nm.

The time needed for biosynthesis of the protein is:

$1395/20 = 69.7 \approx 70$ s, that is a bit more than one minute.

22.8 Taking into account that the A:C ratio is 1:5, the probability of finding A and C at any position is 1/6 and 5/6, respectively. Thus, the probability of finding certain codons is:

$$AAA = (1/6)^3 = 1/216$$

$$CCC = (5/6)^3 = 125/216$$

$$AAC = (1/6)^2 * 5/6 = 5/216$$

$$CCA = (5/6)^2 * 1/6 = 25/216$$

$$ACA = 1/6 * 5/6 * 1/6 = 5/216$$

$$CAC = 5/6 * 1/6 * 5/6 = 25/216$$

$$ACC = 1/6 * (5/6)^2 = 25/216$$

$$CAA = 5/6 * (1/6)^2 = 5/216$$

Using the table of genetic code one gets: Lys:Asn:Thr:Pro:His:Gln=1:5:30:150:25:5

22.9 Anticodon has no influence on the CCA3' terminus. Thus, the mutant tRNA will add tyrosine to the positions where serine was initially expected with respect to mRNA sequence. This may lead to improper folding of the protein and total or partial loss of its functional activity.

22.10 Glu is encoded by GAA and GAG, and His by CAU and CAC. Two substitutions (of the 1st and 3rd residues) are needed to make this mutation true, which is quite improbable. Single residue mutations occur much more frequently, and Glu to Gln mutation can serve as an example (together with many other mutations of this type).

THEORETICAL 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.

23.1 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).

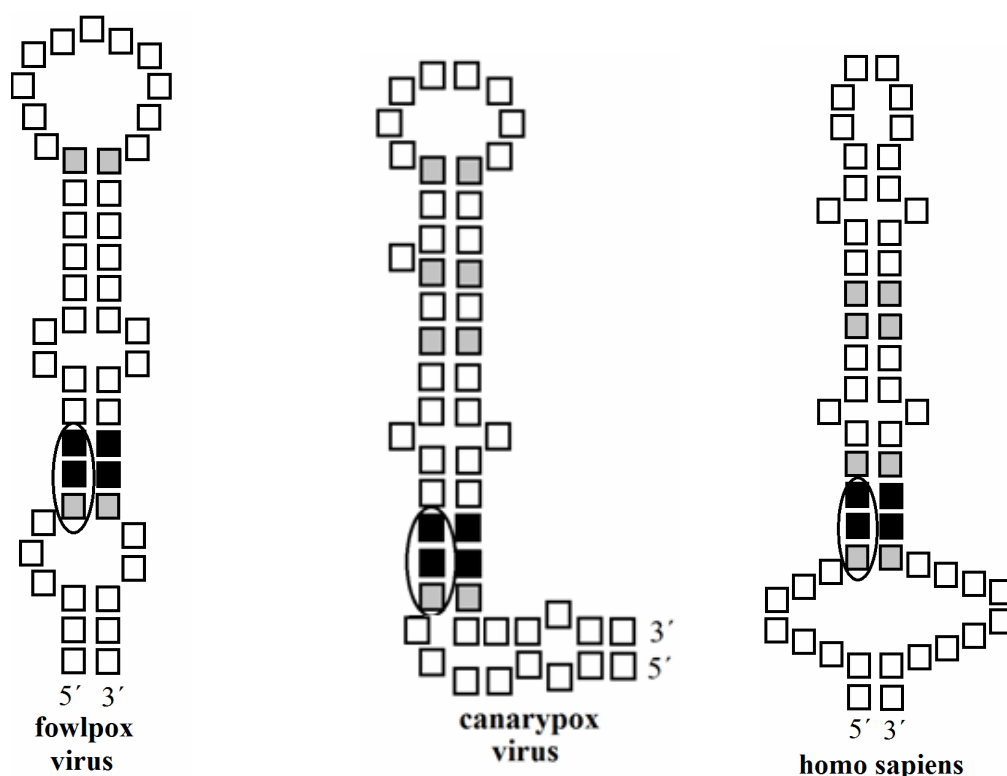
23.2 How many individual peptides are in agreement with the above information?

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 dm³ of gas (STP – standard temperature and pressure).

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

23.4 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	...GCUGC ^X AAUGAAGAAAUGACUAUAAAUAGAUGGGUCAUGCCUGACACGCAAAG...
2	...AGGCACUCAUGACGGCCUGCCUGCAAACCUGCUGGGUGGGGCAGACCCGAAAAUCCAC ...
3	...GACGAGAUAAUGAAGAAAUGGUCCUAAACAGAUGGGUCGUUCCUGACACCCCGG...

- 23.5** 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.
- 23.6** Draw the unusual base pair guanine-uracil found in the hairpin structure, and show the hydrogen bonds.
- 23.7** 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.

No	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.

- 23.8** 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.

SOLUTION OF PREPARATORY PROBLEM 23

- 23.1** If **X** is an acyclic dipeptide, **A** and **B** should be composed of 28 atoms in total (25+3 for H₂O). In the case of an acyclic tripeptide similar calculations lead to 31 atoms in total (25+6 for 2 H₂O), this being true for any of two combinations of residues in the tripeptide (A+2B or 2A+B). Analysis of the structures of all proteinogenic amino

acids given in Wikipedia suggests glycine as one with the minimal number of atoms (10) followed by alanine formed by 13 atoms. Thus, the tripeptide with the minimal number of atoms is composed of 2 glycines and 1 alanine. The total number of atoms (33) in the amino acids forming this tripeptide exceeds 31, which makes any tripeptide as well as large peptides impossible. Therefore, X is a dipeptide.

23.2 Both α -carboxylic and α -amino groups exist mostly in the ionic forms at pH 4.7. Ionization state of the side groups at the given pH value should be determined individually based on their pK_a values as reported in Wikipedia. One should leave into consideration only amino acids with the number of atoms less than 19 ($28-10=18$; this is maximal possible value in case one of two amino acids is glycine). Surprisingly, the data found on different Wikipedia pages lead to contradictory results. According to the former weblink:

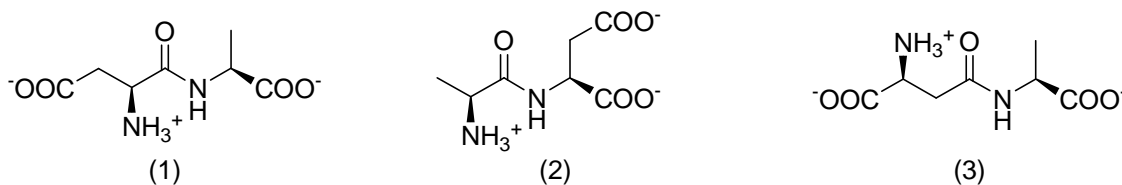
(http://en.wikipedia.org/wiki/Proteinogenic_amino_acid),

only ten amino acids can be further considered. These are:

Amino acid	Prevailing form at pH 4.7 (according to Wikipedia)	Number of atoms	Amino acid	Prevailing form at pH 4.7 (according to Wikipedia)	Number of atoms
Gly		10	Asp		15
Ala		13	Pro		17
Cys		14	Thr		17
Sec		14	Asn		18
Ser		14	Glu		18

The listed amino acids provide for the following dipeptides (without regard to N- and C-termini): Ser-Cys, Ser-Sec, Cys-Sec, Gly-Asn, Gly-Glu и Asp-Ala. Taking into

account the residue positioning (N- or C-terminal), one gets two different dipeptides for each of 4 former pairs, and 3 dipeptides for each 2 latter pairs (note that β -carboxyl group of Asp and γ -carboxyl group of Glu can be also involved in peptide bond formation; see an example below).



Thus, the total number of dipeptides equals to 14. However, serious caution is needed when using Wikipedia, since it is a collection of the user-generated content. Note that pKa values of some groups are absolutely incorrect (section "Side Chain Properties"). In particular, the side group of Asn is absolutely non-protonated at pH 4.7. Finally, the correct number of individual peptides is 12 (excluding Gly-Asn and Asn-Gly).

Screenshot of the webpage http://en.wikipedia.org/wiki/Proteinogenic_amino_acid dated 20.10.2012 is given below. Being irresponsible of these mistakes, authors of the problem promise to correct the data after publishing the Solutions to Preparatory problems.

Amino acid	Short	Abbrev.	Side chain	Hydrophobic	pKa	Polar	pH	Small	Tiny	Aromatic or Aliphatic	van der Waals volume
Alanine	A	Ala	-CH ₃	X	-	-	-	X	X	-	67
Cysteine	C	Cys	-CH ₂ SH	-	8.18	-	acidic	X	X	-	86
Aspartic acid	D	Asp	-CH ₂ COOH	-	3.90	X	acidic	X	-	-	91
Glutamic acid	E	Glu	-CH ₂ CH ₂ COOH	-	4.07	X	acidic	-	-	-	109
Phenylalanine	F	Phe	-CH ₂ C ₆ H ₅	X	-	-	-	-	-	Aromatic	135
Glycine	G	Gly	-H	X	-	-	-	X	X	-	48
Histidine	H	His	-CH ₂ -C ₃ H ₃ N ₂	-	6.04	X	weak basic	-	-	Aromatic	118
Isoleucine	I	Ile	-CH(CH ₃)CH ₂ CH ₃	X	-	-	-	-	-	Aliphatic	124
Lysine	K	Lys	-(CH ₂) ₄ NH ₂	-	10.54	X	basic	-	-	-	135
Leucine	L	Leu	-CH ₂ CH(CH ₃) ₂	X	-	-	-	-	-	Aliphatic	124
Methionine	M	Met	-CH ₂ CH ₂ SCH ₃	X	-	-	-	-	-	-	124
Asparagine	N	Asn	-CH ₂ CONH ₂	-	5.41	X	weak basic	X	-	-	96
Proline	P	Pro	-CH ₂ CH ₂ CH ₂ -	X	-	-	-	X	-	-	90
Glutamine	Q	Gln	-CH ₂ CH ₂ CONH ₂	-	-	X	weak basic	-	-	-	114
Arginine	R	Arg	-(CH ₂) ₃ NH-C(NH) ₂	-	12.48	X	strongly basic	-	-	-	148
Serine	S	Ser	-CH ₂ OH	-	5.68	X	weak acidic	X	X	-	73
Threonine	T	Thr	-CH(OH)CH ₃	-	5.53	-	weak acidic	X	-	-	93
Selenocysteine	U	Sec	-CH ₂ SeH	-	5.73	-	acidic	X	X	-	-
Valine	V	Val	-CH(CH ₃) ₂	X	-	-	-	X	-	Aliphatic	105
Tryptophan	W	Trp	-CH ₂ C ₈ H ₆ N	-	5.885	-	weak basic	-	-	Aromatic	163
Tyrosine	Y	Tyr	-CH ₂ -C ₆ H ₄ OH	-	10.46	X	weak acidic	-	-	Aromatic	141

Note: The pKa values of amino acids are typically slightly different when the amino acid is inside a protein. Protein pKa calculations are sometimes used to calculate the change in the pKa value of an

At the same time, the pKa values found at the other webpage (http://en.wikipedia.org/wiki/Amino_acid) are correct.

- 23.3** One should analyze all five variants of dipeptides (with no regard to N- and C-termini) from i. 2 by calculating masses of corresponding precipitates. Typical procedure is given below for the correct answer (Cys-Sec):



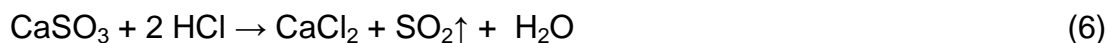
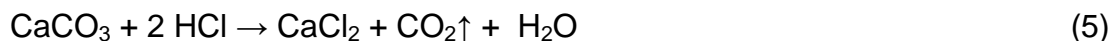
Amount of substance of dipeptide:

$$\frac{1.000 \text{ g}}{271.19 \text{ g mol}^{-1}} = 3.687 \text{ mol}$$

Thus, the mass of precipitate is:

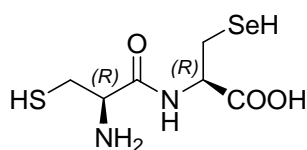
$$m(\text{precipitate}) = 3.687 \cdot 10^{-3} \text{ mol} \times (6 \times 100.09 + 120.14 + 167.04) \text{ g mol}^{-1} = 3.273 \text{ g}$$

However, further calculations according to the equations of chemical reactions of precipitate dissolution in hydrochloric acid

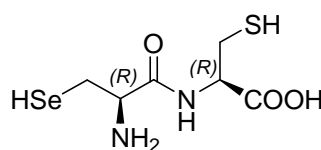


provide for contradictory results. Gas volume given in the task is by approx. 15 % less than that obtained from the calculations. The only reason behind the difference is the deficiency of hydrochloric acid with respect to the precipitate amount (Note that by contrast to the rest of the task, there is no indication of an excess or deficiency in the case of hydrochloric acid!).

Since the available data is insufficient to decide on the sequence of amino acid residues, both Cys-Sec and Sec-Cys are accepted as correct answers for X.



(1)

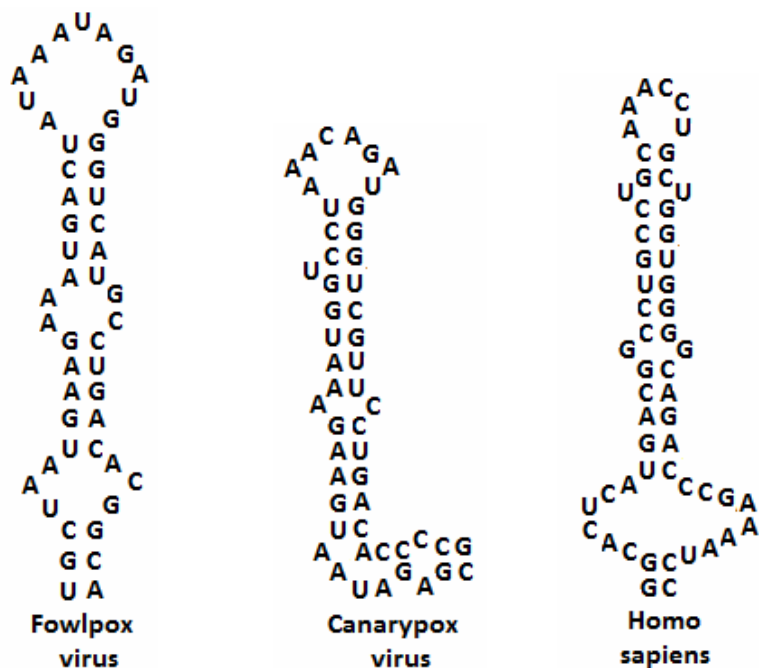


(2)

- 23.4** The –SeH group is a much stronger reducing agent than the –SH group. Thus, Sec is very readily oxidized, which makes its presence as free selenocysteine inside a cell impossible.
- 23.5** Searching for a correlation between the given images and sequences is much easier than can be expected. There could be many ways to reach the correct answer. A sample strategy is given below. First, one should decide which of the fragments refers to human RNA. Genomes of the viruses belonging to the same family should be phylogenetically close, with a slight divergence from the common ancestor. Indeed, sequences 1 and 3 reveal high similarity, both dramatically differing from sequence 2, the latter thus being attributed to human cell. Next step is the search for nucleotides corresponding to the black boxes in the image of human RNA. Note that there are colorless and grey boxes to the ends from black ones. These include 9 nucleotides at the 5'- and 11 nucleotides at the 3'-end. These forbidden areas are highlighted red in the hereunder sequence. Nucleotides corresponding to the black boxes are located between the red fragments, and should be twice two consecutive purine nucleotides AG, AA or GG (all options highlighted yellow). Furthermore, there should be exactly 30 nucleotides between the yellow fragments, which allows the final assignment (highlighted yellow and underlined).

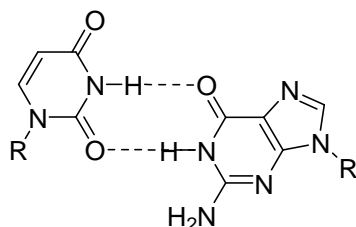
AGGCACUCAUGACGGCCUGCCUGCAAACCUGCUGGUGGGGCAGACCCGAAAAUCCAC

Thus, the encircled codon is UGA, which can be also found in fragments 1 and 3. Using the above strategy, one can fill in the rest two images and find the correlation



between the images and fragments (fragments 1, 2, and 3 refer to the images of the fowlpox virus, homo sapiens, and canarypox virus, respectively).

23.6 Guanine-uracil is the so-called Wobble Base Pair.



23.7 UGA, according to the table of genetic code, is known as the STOP codon terminating translation. However, it is stated in the problem that the chain elongation proceeds after UGA (variants 2 and 3 invalid). UGA is similarly located in sequences of very dissimilar organisms (a mammal and viruses), which underlines its importance for translation and makes variant 5 hardly possible. Variant 4 can be also discriminated, since translation is an uninterrupted process.

Thus, variant 1 is the correct answer. Indeed, UGA in a certain motive (referred to as **SECIS element**, **Selenocysteine Insertion Sequence**) is read as the codon determining selenocysteine inclusion into polypeptides. In viruses, SECIS element is located in the translated region of RNA. In eukaryotes, this hairpin-like structure is found in the unreadable part of mRNA (in 3'-untranslated region, 3'-UTR), and Sec is not found in human proteins.

- 23.8** Knowledge of the UGA position allows setting the reading frame. In principle, there could be various mutations meeting the requirements. Examples are given below. Choosing a mutation, one should keep in mind that the wild type and mutant codons must encode the same amino acid. Also, nucleotides of this codon should not be involved in maintaining the secondary structure of SECIS element (no hydrogen bonding to opposite nucleotides). Thus, one can suggest U-23→C-23 mutation for the fowlpox virus (both are tyrosine codons), and A-28→G-28 mutation for the canarypox virus (both are lysine codons).
-
-

THEORETICAL 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 molar mass of less than 250 g mol^{-1} 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: $N_{\text{nitrogen}}(\mathbf{B}) \geq N_{\text{nitrogen}}(\mathbf{A})$.

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

24.2 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.

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

24.4 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

24.5 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**.

24.6 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**.

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

24.8 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 known that this amino acid does not have its own transfer RNA (tRNA).

24.9 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.

No	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

SOLUTION OF PREPARATORY PROBLEM 24

24.1 Calculation of molar ratios of carbon, hydrogen and oxygen in **A** – **C** allows determining their minimal molar masses corresponding to the net formulae (note that isotopic ratios of C, H, N and O are native):

Compound	Calculation of ratios	Calculation of minimal molar mass (g mol ⁻¹)
A	$n(\text{C}) : n(\text{H}) : n(\text{O}) = \frac{31.09}{12.01} : \frac{5.74}{1.008} : \frac{16.57}{16.00} = 5 : 11 : 2$	$M = \frac{60.05 \times 100}{31.09} = 193.1$
B	$n(\text{C}) : n(\text{H}) : n(\text{O}) = \frac{26.67}{12.01} : \frac{5.04}{1.008} : \frac{17.77}{16.00} = 4 : 9 : 2$	$M = \frac{48.04 \times 100}{26.67} = 180.1$
C	$n(\text{C}) : n(\text{H}) := \frac{9.24}{12.01} : \frac{3.10}{1.008} = 1 : 4$	$M = \frac{12.01 \times 100}{9.74} = 130.0$

With provision of the upper bound ($M < 250 \text{ g mol}^{-1}$), true and minimal molecular weights coincide. The residual molecular weights available for the other two elements (besides C, H, and O) in **A** and **B** are of 90.0 and 91.0 g mol⁻¹, respectively. There are two possible reasons behind the difference in the residual molar masses for **A** and **B** ($91.0 - 90.0 = 1 \text{ g mol}^{-1}$). These are dissimilarity of

atomic masses of the fifth elements in **A** and **B** and/or different number of nitrogen atoms in these compounds. All possible variants of the number of nitrogen atoms (cannot exceed 6) in **A** are considered in the hereunder table:

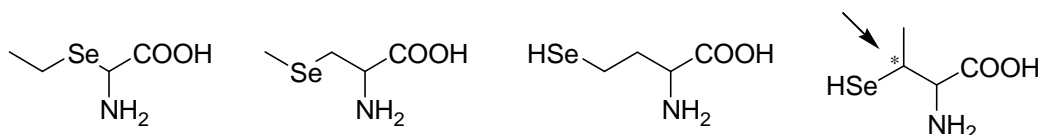
Number N of atoms in A	Residual molar mass left for the 5th element in A	Variants of the 5 th element	Biochemical sense
1	76	-	-
2	62	2 P	To be considered
3	48	1 Ti?	Impossible
		2 Mg?	Impossible
		3 O?	Impossible
		4 C?	Impossible
4	34	-	-
5	20	1 Ne?	Impossible
6	6	6 H?	Impossible

With provision of the inequation given in the problem text, the variant of 2 nitrogen atoms in **A** corresponds to 1 or 2 nitrogen atoms in **B**, and 75 or 63 g mol⁻¹ left for the 5th element in the latter compound, respectively. No reasonable variants are in agreement with the above values. Therefore, we seem to have come up against a brick wall.

24.2 Difference by 1 g mol⁻¹ in the molecular weights of the 5th element in **A** and **B** is left as the only reason. This can be true in case of isotopes (note that native isotope ratios are mentioned only for four elements!). If so, isotopes should be stable (stability of all initial compounds) and most likely of one and the same element (**A**, **B**, and **C** are precursors of the same compound **X**). With account of the equal number of nitrogen atoms in **A** and **B**, the following set of isotope combinations is available: 20-21, 34-35, 48-49, 62-63, 76-77. Furthermore, the difference of 1 g mol⁻¹ unambiguously suggests only one atom of the 5th element in each of **A** and **B**.

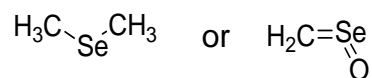
Two sets of stable isotopes (⁴⁸Ti-⁴⁹Ti and ⁷⁶Se-⁷⁷Se) formally fit well. Since there are no native titanium-containing amino acids, the elemental composition of **A** and **B** is finally found as: C, H, N, O, and Se.

24.3 As determined above, the molecular formula of **B** is $C_4H_9SeNO_2$. Four structures can be proposed for this α -amino acid. The rightmost structure contains two chiral atoms, thus being invalid, whereas the leftmost one is unstable. So, two central structures are left as the correct answer.



24.4 Both *R*- and *S*-amino acids are found in nature. Since it is not mentioned in the problem text which exactly of **A** and **B** is found in proteins, it is impossible to unambiguously assign configurations of α -carbon atoms without additional information.

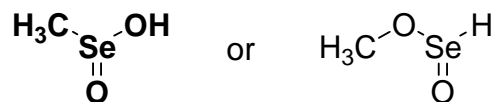
24.5 Gases **A1**, **B1**, and **C1** have molar masses of 106, 107 and 112 $g\ mol^{-1}$, respectively. It is seen that the difference in the molecular weights of **A** and **B** ($1\ g\ mol^{-1}$) is retained for their metabolites. Thus, **A1** and **B1** are likely to be isotopologues. Besides selenium, **A1** and **B1** contain elements with a total residual molecular weight of $106 - 76 = 30\ g\ mol^{-1}$. Since gaseous metabolites contain hydrogen, there are two possible variants of their molecular formula: C_2H_6Se or CH_2SeO . With provision of identity of hydrogen atoms in **A1**, the following structures are possible:



Of these two, only dimethylselenide does not contain π -bonds. Finally, **A1** is $(CH_3)_2^{76}Se$, and **B1** is $(CH_3)_2^{77}Se$.

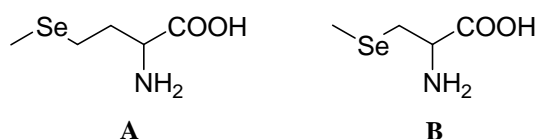
24.6 The atomic weight of selenium isotope in **C1** is $76 + (112-106) = 82$ a.u. (Note that the final metabolite is the same for all three initial original compounds!). Residual molecular weight left for the 4th element in **C** (it consists of only four elements) is $130 - 16 - 82 = 32\ g\ mol^{-1}$, which corresponds to two atoms of oxygen. Thus, the molecular formula of **C** is $CH_4O_2^{82}Se$.

Presence of methyl groups in **C1** as well as lack of C-O bonds in the structure allow the final ascertainment of the structural formula of **C** (the leftmost of the hereunder ones with ⁸²Se):



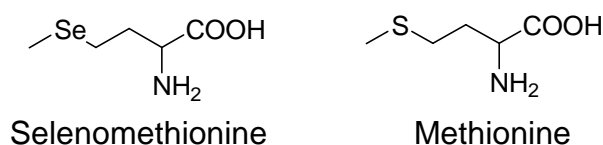
Then, **X** is methylselenide CH_3SeH , and **C1** is $(\text{CH}_3)_2\text{Se}$ produced as result of **X** methylation (transferase reaction).

24.7 As determined in i. 6, methylation is the second step of the processes under consideration. With respect to extremely high specificity of enzymes, all substrates subjected to methylation should be very similar. Thus, the isotopologues of **X** ($\text{CH}_3^{76}\text{SeH}$ and $\text{CH}_3^{77}\text{SeH}$) are the only reasonable intermediates on the way from **A** and **B** to **A1** and **B1**, respectively. These intermediates can directly originate only from compounds containing $\text{CH}_3\text{-Se-}$ residue. Thus, selenomethionine and methylselenocysteine can be attributed to **A** and **B**:



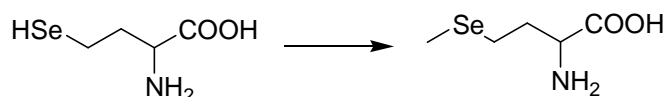
24.8 Since the experiment under discussion is aimed at revealing pathways of selenium metabolism, it is reasonable to check masses of selenium in each of the administered compounds. Calculations involving the molecular weights of **A**, **B**, and **C** and masses of these compounds in the mixture provide for a wonderful result: the mixture contains 25 μg of each of selenium isotopes.

24.9 Variant 2 is the correct choice. Selenomethionine is structurally similar to methionine (compare the structures hereunder), which sometimes leads to mistakes in translation and false insertion of selenium-containing amino acid instead of sulphur-containing one.



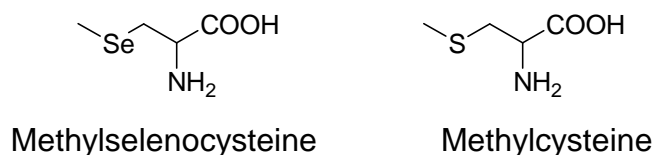
Isotope ^{76}Se is found in nature (~1% of the total selenium pool), so the residue of selenomethionine with ^{76}Se can be found (though rarely) in proteins.

Variant 1 is impossible, since posttranslational modification leading to **A** should involve methylation of selenohomocysteine residue, the latter amino acid also lacking its own tRNA:



Variants 3 and 5 are impossible, since protein biosynthesis admits the only way of polypeptide chain elongation, which involves an amino acid residue transfer from aminoacyl-tRNA.

Variant 4 is impossible for the same reasons as Variant 2. Methylselenocysteine is structurally similar to S-methylcysteine (compare the hereunder structures), which is not a canonical amino acid, thus lacking its own tRNA:



THEORETICAL 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:



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.

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

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

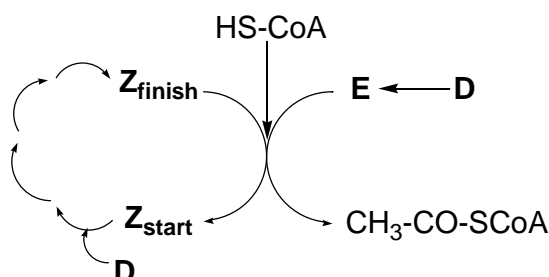
25.3 Choose the appropriate $l : m$ ratio for the reaction (1) from the variants given below.

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.

25.4 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:



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

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

25.5 Determine the total number of atoms in Z_{start} and 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 ($M = 809.6 \text{ g mol}^{-1}$) were detected in the experiment.

25.6 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 Work out the formulae composed of only guanine (G) and cytosine (C) present in equimolar quantities and randomly positioned.

25.7 What is the ratio between the amino acid residues in the oligopeptide 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.

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

SOLUTION OF PREPARATORY PROBLEM 25

25.5 Glucose consists of carbon, oxygen and hydrogen. As a result of its fermentation in H₂O the following gaseous (at STP) products could be theoretically formed:

- 1) Molecular hydrogen,
- 2) Various hydrocarbons,
- 3) Formaldehyde,
- 4) CO and CO₂.

Absence of C-H bonds in **C** and **D** allows excluding variants 2 and 3 from further consideration.

Molecular mass of the gas mixture is $10.55 \times 2 \text{ g mol}^{-1} = 21.1 \text{ g mol}^{-1}$. It is obvious that hydrogen is one of the two gases, whereas either CO or CO₂ is the other one. CO seems to be an improbable variant; still all the options should be checked by applying the hereunder formula for n :

$$M(\mathbf{C}) = \frac{n}{n+10} + M(\mathbf{D}) \frac{10}{n+10} = 21.1$$

$$n = \frac{211 - 10 m(\mathbf{D})}{M(\mathbf{C}) - 21.1}$$

C	D	<i>Coefficient n</i>
H ₂	CO ₂	12.0
CO ₂	H ₂	8.3
H ₂	CO	9.6
CO	H ₂	27.7

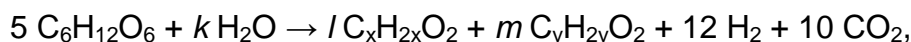
Since n is integer in only one case, **C** and **D** are attributed to H₂ and CO₂, respectively.

Note that bacterial cultures exist in specific, sometimes solid, nutritious media. Thus, conventional data of gases (in particular, of CO₂) solubility in water may be inapplicable.

25.2 With respect to the results in 25.1, the updated reaction (1) is rewritten as:



- a) In the case when each of A and B is a saturated monocarboxylic acids, the equation transforms into:



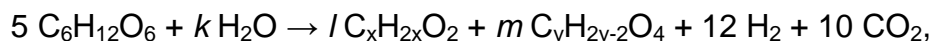
where x and y are the numbers of carbon and hydrogen atoms in **C** and **D**, respectively.

With account of the balance of the elements numbers, one gets the hereunder system of equations:

Element	Balance equation
C	$l \cdot x + m \cdot y = 20$
H	$18 + k = l \cdot x + m \cdot y$
O	$k = 2l + 2m - 10$

It is seen from the first two equations that $k = 2$. Thus, the equation for oxygen can be rewritten as $l + m = 6$

- b) In the case when **A** is a saturated monocarboxylic and **B** a saturated dicarboxylic acids (reverse variant is equivalent), the equation transforms into:



where x and y are the numbers of carbon and hydrogen atoms in **C** and **D**, respectively.

Further analysis provides an analogous system of equations:

Element	Balance equation
C	$l \cdot x + m \cdot y = 20$
H	$18 + k = l \cdot x + m \cdot y - m$
O	$k = 2l + 4m - 10$

There is only one set of integer values corresponding to $m = k = 1$. Still, then $l = 3.5$, which is in contradiction with the conditions of the problem.

A and **B** with higher number of carboxylic groups (for example, two dicarboxylic acids) are impossible, as this results in negative k , l , or m .

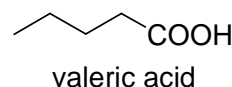
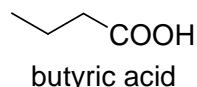
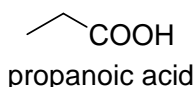
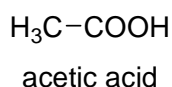
25.3 l and m are integers, and $l + m = 6$. This suggests the following possible ratios: 1 : 1 (3 : 3), 1 : 2 (2 : 4) and 1 : 5. Still, $l \cdot x + m \cdot y = 20$, which makes the ratio of 1 : 1 impossible (both x and y non-integer, $20 / 3 = 6.67$). Ratios of 2 : 1 and 5 : 1 are theoretically possible. Thus, the correct variants are b, e and f.

25.4 The next step is a search for integer solutions of the equation $l \cdot x + m \cdot y = 20$ for the ratios established in i. 3.

$L = 2; m = 4$		$l = 1; m = 5$	
x	y	x	y
8	1	15	1
6	2	10	2
<u>4</u>	<u>3</u>	<u>5</u>	<u>3</u>
	<u>2</u>	<u>4</u>	

Since the number of carbon atoms decreases as a result of fermentation ($x < 6$ and $y < 6$), only the variants underlined in the above table are left for consideration.

These correspond to four unbranched monocarboxylic acids:



Further discrimination of the variants based on the available data is impossible.

For your information: **A** and **B** are acetic and butyric acids, respectively.

25.5 Since $\mathbf{Z}_{\text{start}}$ and $\mathbf{Z}_{\text{finish}}$ contain the same number of nitrogen atoms, a system of equations (2) and (3) can be set up:

$$\frac{a}{b} = 0.12727(2); \quad \frac{a}{b+n} = 0.12069(3)$$

$$b = 18.34 n$$

where a is the number of N atoms, whereas b and $b+n$ are the total numbers of atoms in $\mathbf{Z}_{\text{finish}}$ and $\mathbf{Z}_{\text{start}}$, respectively.

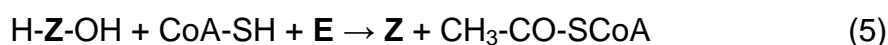
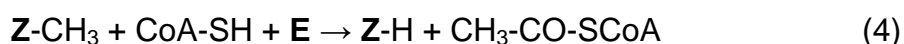
The given limitation of less than 100 atoms in each of $\mathbf{Z}_{\text{start}}$ and $\mathbf{Z}_{\text{finish}}$ can be written as $n < 6$. Variable b is necessarily integer, thus leading to the solely possible combination of $b = 55$ and $n = 3$. So, $\mathbf{Z}_{\text{start}}$ and $\mathbf{Z}_{\text{finish}}$ are composed of 58 and 55 atoms, respectively. This means that $\mathbf{Z}_{\text{start}}$ loses 3 atoms in acetyl-CoA formation.

25.6 The difference in the number of hydrogen atoms in Z_{start} and Z_{finish} is:

$$\Delta N_{\text{H}} = N_{\text{H}}(Z_{\text{start}}) - N_{\text{H}}(Z_{\text{finish}}) = 58 \times 0.43103 - 55 \times 0.41818 = 2$$

Thereby, two of three atoms appearing in acetyl-CoA from Z_{start} are hydrogen atoms. Oxygen or carbon can be the third atom lost by Z_{start} . In the former case, Z_{start} loses H_2O , and in the latter case CH_2 -group, which is formally equivalent to substituting a CH_3 -group with 1 hydrogen atom.

Both variants can be rewritten in a form of equations (4) and (5):



Equation (5) is invalid with any \mathbf{E} , whereas equation (4) is correct, if \mathbf{E} is carbon monoxide CO formed via enzymatic reduction of CO_2 .

Since bacteria cultivation proceeds in the presence of isotope-labeled CO_2 , the number and isotope distribution of nitrogen atoms in acetyl CoA are not influenced.

Thus, the molecular mass of acetyl-CoA isotopologues is:

$$M(\text{labeled AcCoA}) = \frac{100 \times 14.01 \times 7}{12.08} = 811.8 \text{ g mol}^{-1}$$

Molecular mass of unlabeled acetyl-CoA is 809.5. With account of rounding of nitrogen mass fractions, the difference is of 2 g/mol. Two hereunder variants are possible:

- 1) CO_2 labeled with ^{13}C enters the reaction, thus giving acetyl residue with two ^{13}C atoms;
- 2) CO_2 labeled with two ^{18}O enters the reaction, thus giving acetyl residue with ^{18}O atom.

It is impossible to distinguish between **D1** and **D2** basing on the available data:



The above considered acetyl-CoA biosynthesis is referred to as the Wood-Ljungdahl pathway.

For your information. Exact attributing of **D1** and **D2** is possible using physico-chemical methods of analysis. Both isotopes are stable, which makes the radioactivity based methods useless. The suitable methods include mass-

spectrometry (different patterns are formed for molecular fragments) and ¹³C-NMR spectroscopy (¹⁸O isotope is not used in NMR spectroscopy).

25.7 The initial nucleotide ratio is 1:1, thus the probability of finding G or C at any position equals $\frac{1}{2}$. Hence, the probability of any of eight possible codons is of $\frac{1}{2} \cdot \frac{1}{2} \cdot \frac{1}{2} = \frac{1}{8}$. Four amino acids are each encoded by two codons composed of only G and/or C. Thus, the ratio between Pro, Arg, Gly, and Ala is 1:1:1:1. However, with account of the limited length of the oligopeptide (about 33 amino acid residues), there could be significant deviations from the above ratio. So, variant 6 is the most correct choice.

25.8 Using the table of genetic code, one can write down the nucleotide sequences of the initial and mutant mRNA fragments (see designations of N and N1/N2 in Problem 22.6):

UGG-CAU/C-AUG-GAA/G-UAU/C (initial);
UGG-ACN-UAU/C-GGN-GUN (mutant).

Comparison of two sequences suggests that the mutation (insertion of A) occurred right after the first codon. Mutations influencing polypeptide biosynthesis are classified into two groups: the substitution of base pairs and the frameshift. The latter happens upon deletion or insertion of nucleotides in a number not multiple of three. Then, the initial sequence can be rewritten as:

UGGCAUAUGGAGUAU/C

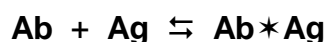
If the mutant protein ends up with the 234rd amino acid residue, the biosynthesis is terminated by a STOP codon present next. Since STOP codons always start with U, the completely deciphered sequence of nucleotides is:

UGGCAUAUGGAGUAU

THEORETICAL 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.



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, metal ions, etc.) can be analyzed by using the same approach.

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

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

26.2 Express \bar{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{n} vs **[Ag]** plot ("titration" curve of Ab with Ag).

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

26.3 a) Plot the Experimental data A (see the table below) as $\frac{[\mathbf{Ab*Ag}]}{[\mathbf{Ag}]}$ vs **[Ab*Ag]**.

b) Express $\frac{[\mathbf{Ab*Ag}]}{[\mathbf{Ag}]}$ 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, ($\times 10^{-6} \text{ mol dm}^{-3}$)	Bound Mg ²⁺ concentration, ($\times 10^{-6} \text{ mol dm}^{-3}$)
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.

- 26.4** a) Derive an expression to be used for determination of the **Ab** valence from the plot analysis in coordinates $[\text{Ab}^*\text{Ag}] / [\text{Ag}]$ vs $[\text{Ab}^*\text{Ag}]$.
- 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 $11 \cdot 10^{-6} \text{ mol dm}^{-3}$.

I total concentration, ($\times 10^{-6}$ mol dm ⁻³)	Free (unbound) I concentration, ($\times 10^{-6}$ mol dm ⁻³)
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

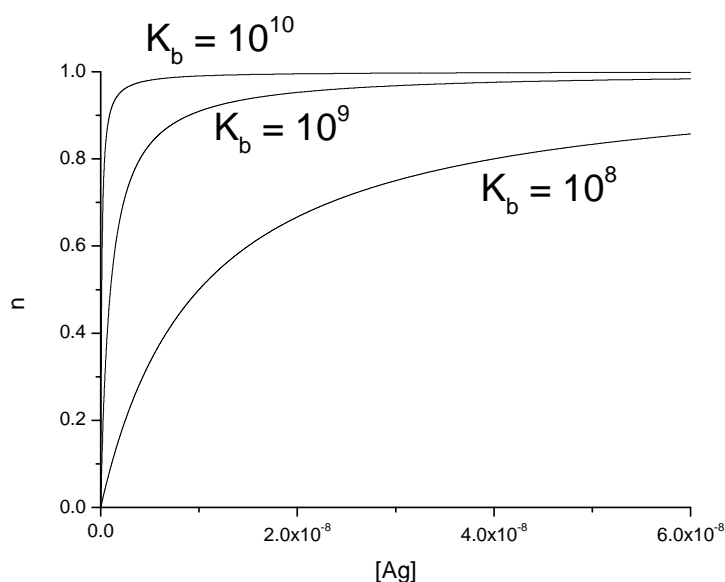
Ab specimen often contains admixtures of other proteins not capable of binding with **Ag**. Thus, a “known” total **Ab** concentration includes both functionally active antibodies and unreactive proteins.

- 26.5** a) Suggest a way for determination of the actual **Ab** concentration from the data analysis in coordinates $[\mathbf{Ab}^*\mathbf{Ag}] / [\mathbf{Ag}]$ vs $[\mathbf{Ab}^*\mathbf{Ag}]$.
- 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?

SOLUTION OF PREPARATORY PROBLEM 26

$$26.1 \quad K_b = \frac{[\text{Ab} * \text{Ag}]}{[\text{Ab}][\text{Ag}]}$$

$$26.2 \quad \bar{n} = \frac{[\text{Ab} * \text{Ag}]}{[\text{Ab} * \text{Ag}] + [\text{Ab}]} = \frac{K_b [\text{Ab}][\text{Ag}]}{K_b [\text{Ab}][\text{Ag}] + [\text{Ab}]} = \frac{K_b [\text{Ag}]}{K_b [\text{Ag}] + 1}$$

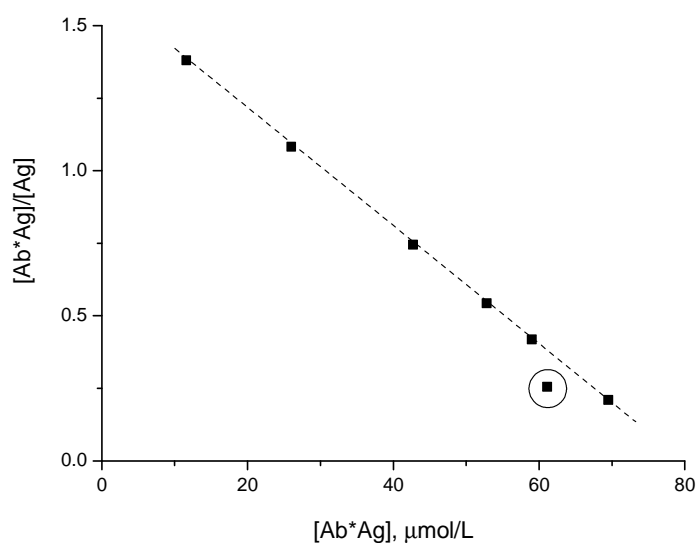


As seen, the titration curves are strongly non-linear, which makes their analysis complicated.

$$26.3 \quad K_b = \frac{[\text{Ab} * \text{Ag}]}{[\text{Ab}][\text{Ag}]} \Rightarrow \frac{[\text{Ab} * \text{Ag}]}{[\text{Ag}]} = K_b (c_{\text{Ab}} - [\text{Ab} * \text{Ag}])$$

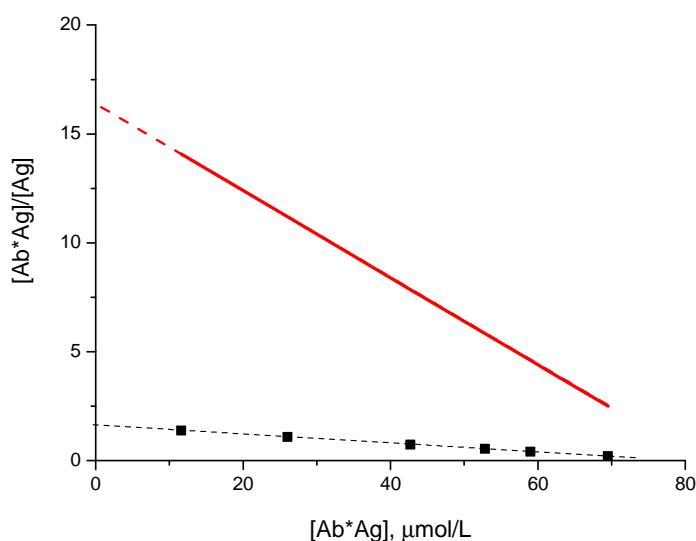
Thus, a plot in such coordinates (referred to as the Scatchard ones) should be a straight line with the slope of $-K_b$ and the intercept of $c_{\text{Ab}}K_b$ (c_{Ab} is the total **Ab** concentration).

This is proved by plotting Set A data, point #6 is seemingly an outlier:



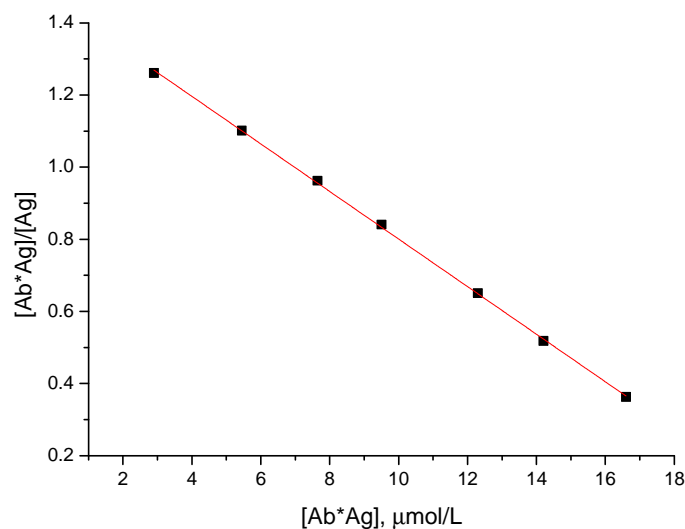
From the experimental data, $K_b = 2 \cdot 10^4$.

According to the above equation, with a 10 times higher K_b , values for both the intercept (1.64 from the original data) and the slope should be 10 times higher:



26.4 If all the binding sites are independent, and K_b does not depend on the fraction of occupied binding sites, mathematically there is no difference between x molecules of antibody with valence N and $N \cdot x$ molecules of antibody with valence 1. Thus, the above mentioned Scatchard equation is only slightly modified to account for several binding sites per antibody:

$$\frac{[\text{Ab} * \text{Ag}]}{[\text{Ag}]} = K_b (N c_{\text{Ab}} - [\text{Ab} * \text{Ag}])$$



The experimental data fit a straight line with a slope of -0.0660 (corresponding to $K_b=6.6 \cdot 10^4$) and an intercept of 1.46 . Thus,

$$1.46 = K_b N c_{Ab} = 6.6 \times 10^4 \times N \times 1.1 \cdot 10^{-5} \Rightarrow N = 2$$

26.5 A clear way to determine C_{Ab} follows from the fact that the K_b value influences both the slope and the intercept of the plot in Scatchard coordinates. As soon as K_b is determined from the slope of the curve, C_{Ab} can be immediately calculated, provided the antibody valence N is known. For instance, for the set A, $N = 1$, $K_b = 2 \cdot 10^4$; $c_{Ab} = 1.64 / 2 \cdot 10^4 \approx 82 \mu\text{mol L}^{-1}$, which reasonably corresponds to the given value of $80 \mu\text{M}$. It can be concluded, thus, that the ADP protein does not contain any functionally inactive antibodies or other impurities.

The same analysis for the set B is impossible, because the real enzyme valence is not known *a priori*. (Value $N = 2$ determined above has been obtained under assumption of 100% enzyme purity.)

THEORETICAL PROBLEM 27

Inorganic polymers: polyphosphates and polysilicones

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

27.1 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.

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

27.3 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 $P_iO_3OH^{(i+1)-}$).

27.4 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, ΔG° 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.

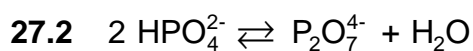
In many cases, the equilibrium constant of a condensation reaction is too low to provide for high-molecular weight products. Other condensation reactions are too fast,

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.

- 27.5** 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?
- 27.6** 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?
- 27.7** 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).

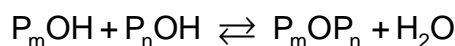
SOLUTION OF PREPARATORY PROBLEM 27

27.1 Well known examples are: C (acetylenic carbon), S (various forms of polymeric sulfur), Se (grey selenium), P (red phosphorus), As (black arsenic), Sb (black antimony). Not all of these substances consist of perfectly linear chain molecules, but for sure these elements are capable of forming quite long polymers.



(ionization state of the phosphate precursor depends on pH).

27.3 With P_i standing for a polyphosphate with the degree of polymerization of i , for the reaction

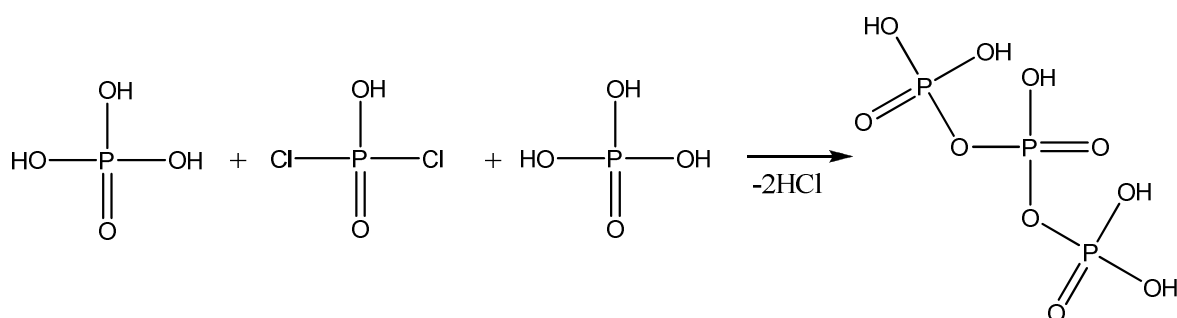


$$K = \frac{[P_{m+n}][H_2O]}{[P_mOH][P_nOH]}$$

As polyphosphates of various degrees of polymerization are not distinguishable, each of concentrations $[P_m]$, $[P_n]$, $[P_{m+n}]$ can be substituted with the total concentration of all phosphate species, thus,

$$K = \frac{[P_{m+n}][H_2O]}{[P_mOH][P_nOH]} = \frac{[H_2O]}{[P_i]}$$

27.4 The following reasons should be taken into account. First, the free energy of hydrolysis is strongly negative, which means that the free energy of condensation (the reverse reaction) is positive. Thus, the equilibrium constant of an elementary condensation stage is low (less than 1), which is not consistent with the high-polymeric phosphate species. In general, lower equilibrium concentration of (poly)phosphate molecules means that more individual condensations have taken place, which is equivalent to the higher average degree of polymerization of the product. This is true for process ii): lower water concentration (at a certain equilibrium constant value) corresponds to lower equilibrium concentration of phosphate molecules (from the expression derived in i. 27.3). Thus, process ii) is more favorable than i). However, process iii) is the most favorable. According to the equation

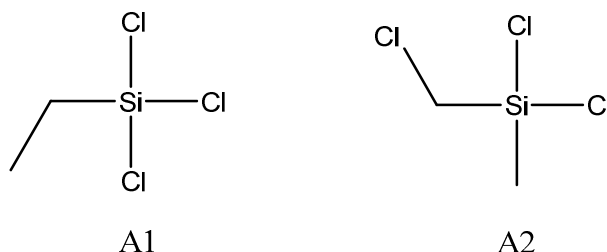


a highly volatile HCl is formed, which is efficiently removed from the reaction mixture by heating. As a result, the equilibrium is shifted rightwards.

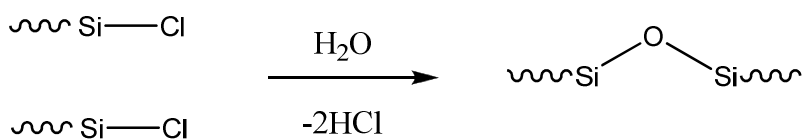
Indeed, only route iii) can be applied in practice for the preparation of polyphosphoric acids. Condensation in concentrated solutions (process ii)) is quite slow, and yields significant amounts of polyphosphoric acids only upon heating (molten H_3PO_4 , 230 - 250°C). Direct condensation in dilute solution (process i)) is so unfavorable that may come true only when coupled with a certain exoergic reaction

(for instance, substrate phosphorylation in various biochemical processes) with the actual mechanism much more complicated than direct condensation.

27.5

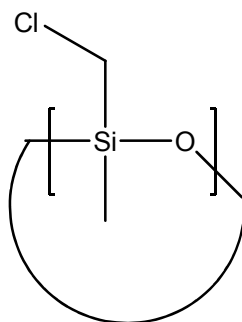


The main chain of the polymer molecule is composed of Si and O atoms:

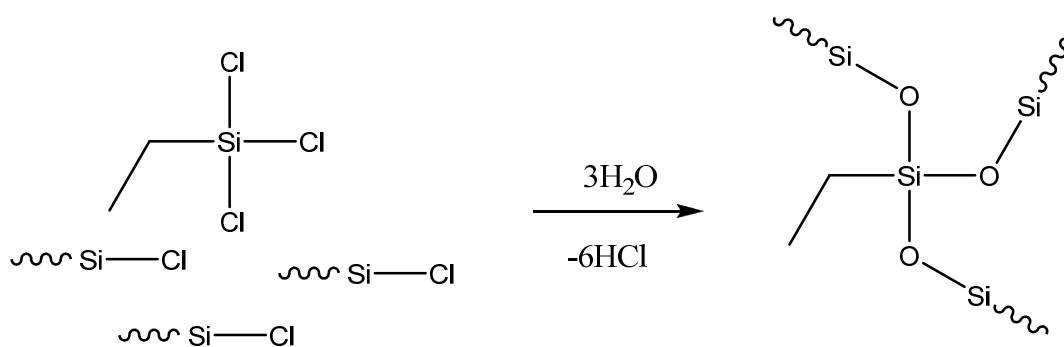


27.6, 27.7

The Si-Cl bond is much more reactive than the C-Cl one in hydrolysis and condensation reactions. Thus, **A2** can be considered bifunctional in polycondensation reaction, giving a non-branched polymer with the cyclic giant macromolecule of poly(chlorodimethylsiloxane) as the final product when absolutely all Si-Cl bonds are reacted:



A1 is trifunctional, thus giving rise to a branched polymer:



If hydrolysis of Si-Cl bonds is incomplete, some Cl residues are present in the polymeric product. Incomplete condensation retains a number of OH-groups in the product.

PRACTICAL PROBLEMS

PREPARATORY PROBLEM 28 (PRACTICAL)

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 $\text{Na}_2\text{H}_2\text{EDTA}$. 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 $\text{Na}_2\text{H}_2\text{EDTA}$. In the second titration, sodium thiosulfate is added to bind the Cu^{2+} ions, thus allowing titration of solely zinc ions with $\text{Na}_2\text{H}_2\text{EDTA}$.

Chemicals and reagents

- Brass sample, ~250 mg per student, *or*
Test solution (a standard solution containing ions Cu^{2+} (about 1.5 g dm^{-3}) and Zn^{2+} (about 1 g dm^{-3}) simulating a digested sample of brass)
- Nitric acid, HNO_3 , concentrated (~70% w/v)
- $\text{Na}_2\text{H}_2\text{EDTA}$ standard solution, $c = 0.0500 \text{ mol dm}^{-3}$
- Acetate buffer solution, $\text{pH} = 5.5 - 6.0$, 0.1 mol dm^{-3} in acetate
- Sodium thiosulfate solution, $\text{Na}_2\text{S}_2\text{O}_3$, ~10% (w/v)
- Metallochromic indicator 4-(2-pyridylazo)resorcinol (PAR), 0.1% aqueous solution (w/v) (0.1% solution of Xylenol orange indicator may be used instead of PAR).

Apparatus and glassware

- Analytical balance (± 0.0001 g)
- Beaker, 10 cm³
- Hotplate
- Volumetric flask, 100 cm³
- Burette, 25 or 50 cm³
- Volumetric pipettes, 2, 5 and 10 cm³
- Erlenmeyer flask, 100 cm³ (3 ea.)
- Graduated cylinders, 10 and 25 cm³

A. Brass digestion

- 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.
- Carefully add 5 cm³ of concentrated nitric acid (the experiment should be done under a fume hood, as NO₂ gas evolves).
- Heat the beaker slightly on a hotplate to provide for an effective dissolution.
- 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.
- Dissolve the contents of the beaker in distilled water, transfer it to a 100.00 cm³ volumetric flask and make it up to the mark.

B. Determination of the total amount of Cu²⁺ and Zn²⁺

- Transfer 10.00 cm³ of the test solution into a 100 cm³ Erlenmeyer flask, add 20 cm³ of water, 5 cm³ of acetate buffer solution and 3 drops of PAR solution, mix thoroughly.
- Titrate the content of the flask with a standard Na₂H₂EDTA solution ($c = 0.0500$ mol dm⁻³) 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 when necessary.

C. Determination of Zn²⁺

- h) Transfer 10.00 cm³ of the test solution into a 100 cm³ Erlenmeyer flask, add 10 cm³ of water, 5 cm³ of acetate buffer solution, 2 cm³ of Na₂S₂O₃ solution and 3 drops of PAR solution, mix thoroughly.
- i) Titrate the content of the flask with 0.0500 mol dm⁻³ standard Na₂H₂EDTA solution until the color changes from red to yellow (for Xylenol orange, the colors are the same).

D. Calculation of Cu²⁺ concentration

- j) The volume of Na₂H₂EDTA which is necessary for Cu²⁺ titration is calculated as the difference of the titrant volumes in titrations **B** and **C**.

Questions and Data Analysis

28.1 Give balanced chemical equations for the reactions that take place when:

- brass dissolves in nitric acid;
- copper and zinc ions are titrated by Na₂H₂EDTA;

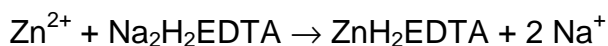
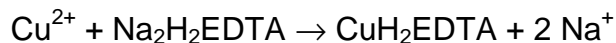
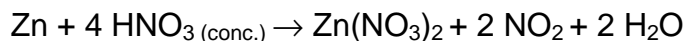
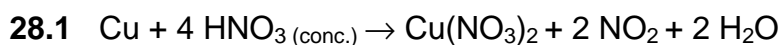
28.2 Explain how Na₂S₂O₃ masks the Cu²⁺ ion, giving the appropriate chemical equation.

28.3 Why should the pH value of the titrated solution be kept within 5 – 6?

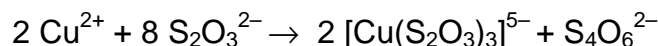
28.4 Calculate the molar fraction of H₂EDTA²⁻ at pH 6. EDTA is a weak acid with the following acidity constants:

$$K_1 = 1.0 \cdot 10^{-2}, \quad K_2 = 2.1 \cdot 10^{-3}, \quad K_3 = 6.9 \cdot 10^{-7}, \quad K_4 = 5.5 \cdot 10^{-11}.$$

28.5 Derive the formulae for calculation of Cu²⁺ and Zn²⁺ concentrations in the test solution. Calculate the mass ratio of Cu and Zn in the alloy.

SOLUTION OF PREPARATORY PROBLEM 28

28.2 Cu^{2+} ions present in the aqueous solution are reduced to Cu^+ by thiosulfate. Moreover, the latter forms with Cu^+ a soluble complex $[\text{Cu}(\text{S}_2\text{O}_3)_3]^{5-}$, which is more stable than $\text{Cu}_2\text{H}_2\text{EDTA}$:



28.3 Metal ions can be titrated with EDTA if the conditional stability constants β' of the metal – EDTA complexes are not less than $10^8 - 10^9$. The β' values are connected with the real constants β as

$$\beta' = \alpha_{\text{EDTA}} \alpha_{\text{M}} \beta,$$

where α_{EDTA} and α_{M} are molar fractions of $\text{H}_2\text{EDTA}^{2-}$ and free metal ion, respectively. As the values of α_{EDTA} and α_{M} significantly depend on pH of the solution, there is an optimal pH range for the titration of metals. In the case of Cu^{2+} and Zn^{2+} , the pH value within 5 to 6 is optimal. In such slightly acidic medium both metals do not form hydroxyl complexes (α_{M} is high), whilst $\text{H}_2\text{EDTA}^{2-}$ is not further protonated (α_{EDTA} is high).

$$\alpha(\text{H}_2\text{EDTA}^{2-}) = \frac{K_1 K_2 [\text{H}^+]^2}{K_1 K_2 K_3 K_4 + K_1 K_2 K_3 [\text{H}^+] + K_1 K_2 [\text{H}^+]^2 + K_1 [\text{H}^+]^3 + [\text{H}^+]^4}$$

$$[\text{H}^+] = 1.0 \cdot 10^{-6}, \quad K_1 = 1.0 \cdot 10^{-2}, \quad K_2 = 2.1 \cdot 10^{-3}, \quad K_3 = 6.9 \cdot 10^{-7}, \\ K_4 = 5.5 \cdot 10^{-11}, \quad \alpha(\text{H}_2\text{EDTA}^{2-}) = 0,59$$

28.5 The first titration (**B**) gives the volume of titrant $V_{\text{Cu+Zn}}$, whilst the second one (**C**) gives V_{Zn} . Zn^{2+} concentration is calculated as follows:

$$\alpha(\text{Zn}^{2+}) (\text{g dm}^{-3}) = V_{\text{Zn}} (\text{cm}^3) \times c_{\text{EDTA}} (\text{mol dm}^{-3}) \times 65.39 \text{ g mol}^{-1} \times 0.1 \text{ cm}^{-3}$$

$$\alpha(\text{Cu}^{2+}) (\text{g dm}^{-3}) = (V_{\text{Cu+Zn}} - V_{\text{Zn}}) \text{ cm}^3 \times c_{\text{EDTA}} (\text{mol dm}^{-3}) \times 63.55 \text{ g mol}^{-1} \cdot 0.1 \text{ cm}^{-3}$$

The mass ratio of the metals in alloy is calculated from $c(\text{Cu}^{2+})$ and $c(\text{Zn}^{2+})$ values in g dm^{-3} :

$$m(\text{Cu}) / m(\text{Zn}) = c(\text{Cu}^{2+}) / c(\text{Zn}^{2+})$$

PREPARATORY PROBLEM 29 (PRACTICAL)

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.

Chemicals

- HNO_3 , solution in water, $\sim 1 \text{ mol dm}^{-3}$
- $\text{NH}_3(\text{aq})$, solution in water, $\sim 1 \text{ mol dm}^{-3}$
- $\text{NaOH}(\text{aq})$, solution in water, $\sim 1 \text{ mol dm}^{-3}$
- NaCl , solid, 0.6 g

Equipment and Glassware

- Conductivity meter
- Analytical balance ($\pm 0.0001 \text{ g}$)
- Burette
- Volumetric pipettes, 10, 15 and 25 cm^3
- Pipette bulb or pump
- Magnetic stirrer
- Stirring bar
- Volumetric flasks, 100 cm^3 (5 each)
- Glass beaker, 100 cm^3

Procedure

- a) Place ammonia and nitric acid solutions into three 100- cm³ 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 (cm ³) of HNO ₃ , (<i>c</i> = 1 mol dm ⁻³)	Volume (cm ³) of HNO ₃ , (<i>c</i> = 1 mol dm ⁻³)
A	10	15
B	10	10
C	20	10

- b) Transfer 25.0 cm³ of solution **A** into a glass beaker using a 25 cm³ transfer pipette.
- c) Titrate the sample solution with a standardized solution of NaOH (~1 mol dm⁻³, known exactly) by adding 0.2 cm³ 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 cm³ of HNO₃ and 10 cm³ of NH₃ 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

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

29.2 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?

- 29.3** 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.
- 29.4** 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 HNO₃ and NH₃.
- 29.5** Using the obtained results, predict the curve shape for the titration of a mixture of sodium hydroxide and free ammonia with HCl.
-

SOLUTION OF PREPARATORY PROBLEM 29

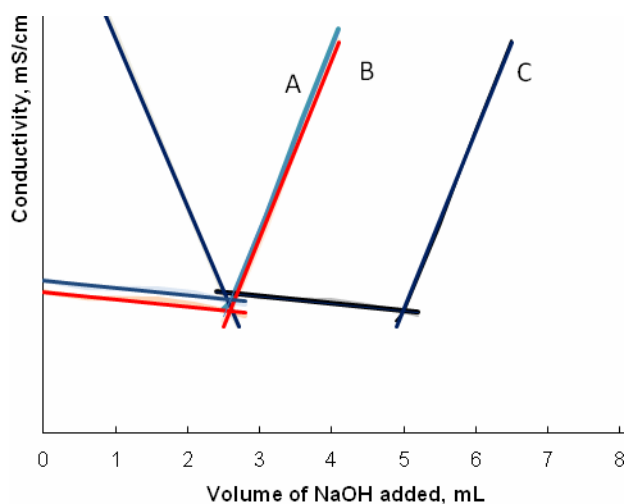
- 29.1** Equilibria in the system can be described by the following equations:



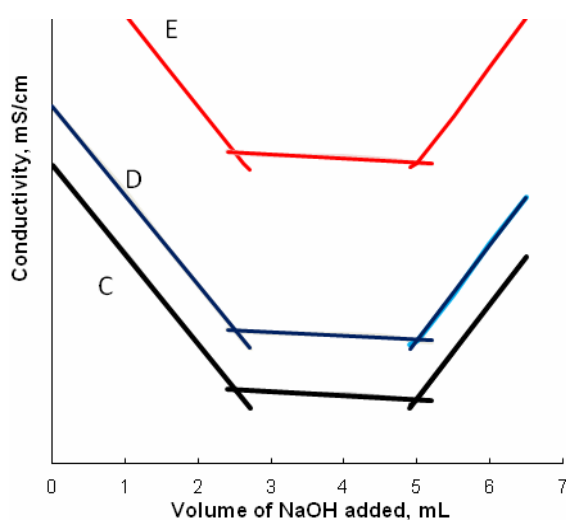
- 29.2** Conductivity of a solution is primarily dependent on the concentration of H⁺ and OH⁻ ions (species with the highest mobility) as well as on that of salts. Solutions **A** and **B** contain the same amount of NH₄NO₃ (solution **A** with an excess of ammonia reveals a bit higher conductivity). On the titration curves, there are monotonously descending portions reflecting the displacement of the weak base (NH₃) from its salt (reaction 2). Minimum conductivity is reached when the concentration of protons appearing from NH₄⁺ hydrolysis is minimal (reaction 2 completed). This is further changed by a sharp rise corresponding to the increasing excess of alkali.

In the case of solution **C**, the first descending portion is steeper (than those for **A** and **B**) and is associated with diminishing concentration of free protons coming from HNO₃. The first equivalence point causes a sharp break of the curve (reaction 1 completed). The second descending portion characterized by a lower slope reflects the displacement of the weak base from its salt (reaction 2). Minimum conductivity is also reached when reaction 2 is completed, which is followed by a sharp rise of conductivity due to the alkali excess.

29.3



Titration of NH_4NO_3 (**A**), of NH_4NO_3 in the presence of an excess of NH_3 (**B**), and of HNO_3 followed by that of NH_4NO_3 (**C**).



Titration of solutions of HNO_3 and NH_4NO_3 diluted with deionized water (**C**), distilled water (**D**), and deionized water containing NaCl (**E**).

The difference between cases **C**, **D**, and **E** is due to various levels of conductivity caused by the salts that are not titrated with NaOH .

29.4 Calculations can be done in the same way as for a regular acid-base titration, using titrant volumes in inflection points $V_{\text{NaOH}(1)}$, $V_{\text{NaOH}(2)}$:

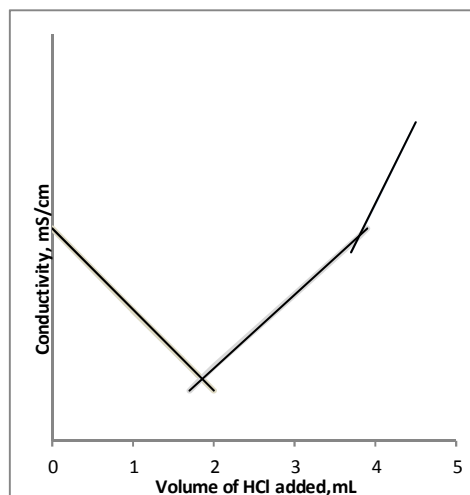
$$c_{\text{H}^+} V_{\text{sample}} = c_{\text{NaOH}} V_{\text{NaOH}(1)}, \quad c_{\text{NH}_4^+} V_{\text{sample}} = c_{\text{NaOH}} \cdot (V_{\text{NaOH}(2)} - V_{\text{NaOH}(1)})$$

Examples.

A and B: If 2.45 cm^3 of NaOH solution ($c = 0.9987 \text{ mol dm}^{-3}$) were spent until the inflection point was reached, then $c_{\text{NH}_4^+} = 0.9987 \times 2,45 = c_{\text{NH}_4^+} \times 25$; $c_{\text{NH}_4^+} = 0.0979 \text{ mol dm}^{-3}$.

C – E: If 2.40 cm^3 of NaOH solution ($c = 0.9987 \text{ mol dm}^{-3}$) were spent until the first inflection point was reached (the neutralization of HNO_3) in a 25.0 cm^3 sample aliquot, then $c_{\text{HNO}_3} = 0.0895 \text{ mol dm}^{-3}$. If the second inflection point was reached at 4.85 cm^3 , then $c_{\text{NH}_4^+}$ is: $0.9987 \times (4.85 - 2.40) = c_{\text{NH}_4^+} \times 25$; $c_{\text{NH}_4^+} = 0.0979 \text{ mol dm}^{-3}$.

29.5. HCl first neutralizes the strong base, which is followed by neutralization of the weak one. So, the titration curve of a mixture of two bases reveals two breaks. NaOH neutralization is accompanied by a linear decrease of conductivity due to lowering concentration of highly mobile hydroxyl ions. After the first equivalence point, conductivity starts increasing due to the formation of a well dissociating salt (a strong electrolyte) as a result of ammonia (a weak electrolyte)



neutralization. After the second equivalence point, conductivity of the solution sharply increases due to the excess of hydrogen ions.

PREPARATORY PROBLEM 30 (PRACTICAL)

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 $(\text{NH}_4)_2\text{HPO}_4$ 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 $(\text{CH}_2)_6(\text{NH}^+)_4$, which is more acidic than the NH_4^+ cation. Another potentiometric titration is necessary to find the total amount of $(\text{CH}_2)_6(\text{NH}^+)_4$, and thus calculate the total amount of diammonium phosphate and ammonium chloride in the sample.

The acidity constants of phosphoric acid:

$$K_{a1} = 7.1 \cdot 10^{-3}, \quad K_{a2} = 6.2 \cdot 10^{-8}, \quad K_{a3} = 5.0 \cdot 10^{-13}.$$

Chemicals and Reagents

- Mixture of $(\text{NH}_4)_2\text{HPO}_4$ and NH_4Cl , about 1 : 1 by mass
- Sodium hydroxide, solution, $c(\text{NaOH}) = 0.1 \text{ mol dm}^{-3}$
- Hydrochloric acid, $c(\text{HCl}) = 0.1 \text{ mol dm}^{-3}$
- Formaldehyde, 20 % CH_2O (aq)

Equipment and Glassware

- Analytical balance ($\pm 0.0001 \text{ g}$)
- Volumetric pipette, 10 cm^3
- Pipette pump

- Burette, 25 cm³
- Beaker, 100 cm³
- Volumetric flask, 100 cm³
- Magnetic stirrer
- Stirring bar
- pH meter

A. Determination of phosphate amount as phosphoric acid

- Weigh about 0.6 g of the test mixture and place it in a 100 cm³ volumetric flask. Fill with water up to the mark.
- Transfer 10 cm³ of the prepared solution into a 100 cm³ beaker using a 10 cm³ volumetric pipette. Add 10 cm³ of hydrochloric acid (0.1 mol dm⁻³, concentration known exactly) using a 10 cm³ volumetric pipette, and dilute it with 20 cm³ of distilled water. Place the beaker onto a magnetic stirrer and put in the stirring bar.
- Titrate the sample with a solution of sodium hydroxide (0.1 mol dm⁻³) adding it by 0.5 cm³ 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.
- 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

- 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.
- Transfer 10 cm³ of the sample solution into a 100 cm³ beaker using a 10 cm³ volumetric pipette. Add 5 cm³ of the formaldehyde solution and wait for 2 min.
- Place the beaker onto the magnetic stirrer and put in the stirring bar. Titrate the sample with a solution of sodium hydroxide (0.1 mol dm⁻³) with constant stirring as described in part A.
- Repeat the titration with new aliquots of the sample solution as needed to obtain consistent results.

Questions and Data Analysis

- 30.1** How many end points are expected during the titration of a mixture of H_3PO_4 and HCl ?
- 30.2** Can color indicators be used in the determination of concentrations of hydrochloric and phosphoric acids in their mixture?
- 30.3** Write down the equations of all the reactions occurred.
- 30.4** Plot the graphs of pH , $\Delta\text{pH} / \Delta V$, and $\Delta^2\text{pH} / \Delta V^2$ 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?
- 30.5** Calculate the content (in weight %) of (a) diammonium phosphate and (b) ammonium chloride in the test sample.
-

SOLUTION OF PREPARATORY PROBLEM 30

30.1 Titration curves for a polyprotic acid (such as phosphoric acid) or a mixture of acids are characterized by more than one endpoint if $K_{a1} : K_{a2} \geq 10^4$ and the equilibrium constant of acidity of the weak acid is more than $n \cdot 10^{-9}$. The equilibrium constants of acidity of phosphoric acid are: $K_{a1} = 7.1 \cdot 10^{-3}$, $K_{a2} = 6.2 \cdot 10^{-8}$, $K_{a3} = 5.0 \cdot 10^{-13}$. Thus, there are two breaks on the titration curve of phosphoric acid (Fig. 1). The third break is not observed due to very low value of K_{a3} .

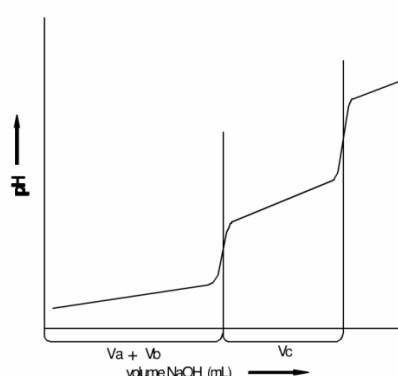
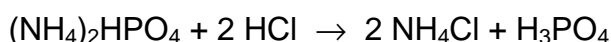


Fig. 1. Titration of a mixture of hydrochloric and phosphoric acids with sodium hydroxide.

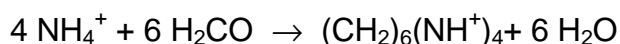
During titration of a *mixture* of hydrochloric and phosphoric acids, the proton of hydrochloric acid and the first proton of phosphoric acid react with sodium hydroxide simultaneously. By the second endpoint H_2PO_4^- is converted into HPO_4^{2-} .

30.2 The first and second equivalence points of H_3PO_4 are observed at pH of about 4.7 and 9.6, respectively. For determination of hydrochloric and phosphoric acids in their mixture, one can use indicators with color change around these pH values (for example, bromocresol green and thymol phthalein for the first and second titrations, respectively).

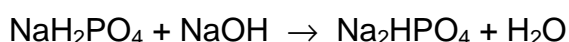
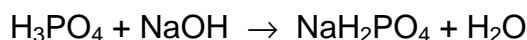
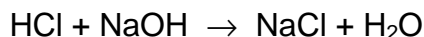
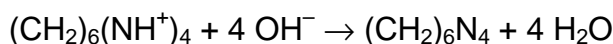
30.3 The following reaction takes place on addition of HCl to the sample:



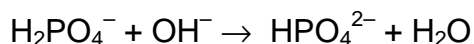
Formaldehyde reacts with ammonium salts to form hexamethylenetetrammonium cation:



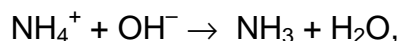
The equations describing the titration of hexamethylenetetrammonium salt, hydrochloric and phosphoric acids with sodium hydroxide:



30.4 A typical analysis of potentiometric titration data is shown in Fig. 2. The most steeply rising portion on the curve (a) corresponds to the endpoint, which can be found more precisely by studying dependences of the first (maximum on curve (b)) or second (zero value on curve (c)) derivatives. In the presence of ammonium salts, the reaction corresponding to the second end point in H_3PO_4 titration



is overlaid by the process



which makes the potential rise gradually rather than sharply (ammonium buffer).

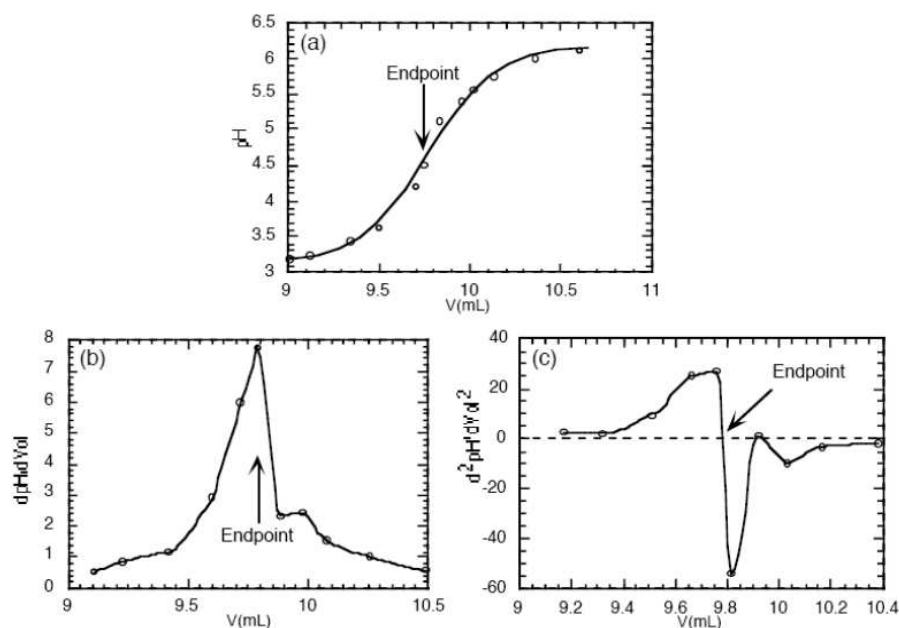


Fig. 2. Typical plots of potentiometric titration: (a) titration curve of an acid with a base; (b) curve of the first derivative; © curve of the second derivative.

30.5 (a) Calculation of phosphate amount

With $V_{\text{NaOH},1}$ designating the volume of sodium hydroxide used in titration **A**, the amount needed to neutralize hydrochloric acid and the first proton of phosphoric acid is:

$$n_{\text{PO}_4} + n_{\text{HCl}} (\text{titrated}) = c_{\text{NaOH}} \times V_{\text{NaOH},1}$$

At the same time,

$$c_{\text{HCl}} \times V_{\text{HCl}} (\text{added}) = n_{\text{HCl}} (\text{titrated}) + 2 n_{\text{PO}_4} \quad (\text{HCl spent for the reaction with } (\text{NH}_4)_2\text{HPO}_4)$$

Then,

$$n_{\text{PO}_4} = c_{\text{HCl}} \times V_{\text{HCl}} (\text{added}) - c_{\text{NaOH}} \times V_{\text{NaOH},1}$$

Since $n_{(\text{NH}_4)_2\text{HPO}_4} = n_{\text{PO}_4}$, one finally gets:

$$w_{(\text{NH}_4)_2\text{HPO}_4} = (10 \times n_{\text{PO}_4} \times M_{(\text{NH}_4)_2\text{HPO}_4}) / m_{\text{mixture}}$$

(b) Calculation of the total amount of diammonium hydrophosphate and ammonium chloride

With $V_{\text{NaOH},2}$ designating the volume of sodium hydroxide used in titration **B** (that is, spent for the neutralization of hexamethylene tetrammonium cation $(\text{CH}_2)_6(\text{NH}^+)_4$ obtained from the ammonium salts), one gets:

$$n_{\text{NH}_4\text{Cl}} + 2 n_{\text{PO}_4} = c_{\text{NaOH}} \times V_{\text{NaOH},2}$$

The amount of phosphate $n_{(\text{NH}_4)_2\text{H}_3\text{PO}_4}$ was determined in experiment **A**, which allows calculating the amount of NH_4Cl

$$n_{\text{NH}_4\text{Cl}} = c_{\text{NaOH}} \times V_{\text{NaOH},2} - 2 (c_{\text{HCl}} \times V_{\text{HCl}} - c_{\text{NaOH}} \times V_{\text{NaOH},1})$$

and its content in the mixture:

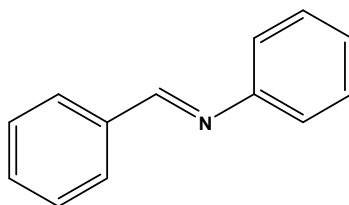
$$w_{\text{NH}_4\text{Cl}} = (10 \times n_{\text{NH}_4\text{Cl}} \times M_{\text{NH}_4\text{Cl}}) / m_{\text{mixture}}$$

PREPARATORY PROBLEM 31 (PRACTICAL)

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 B₆ 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

Equipment and Glassware

- Magnetic stirrer with heating
- Magnetic bar
- Glass beaker, 25 cm³
- Round-bottom two necked flask, 50 cm³
- 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**

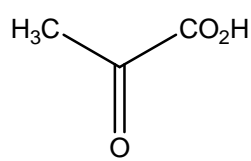
0.42 g of freshly distilled benzaldehyde is placed in a round bottom two necked flask equipped with a reflux condenser and an addition funnel. The reaction vessel is mounted on the magnetic stirrer with a heating mantle. 0.37 g of freshly distilled aniline is poured in the funnel. The aniline is added 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 cm³ glass with 3 cm³ of 96% ethanol. Transfer the reaction mixture from the flask to the glass, wash the flask with 1 cm³ 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

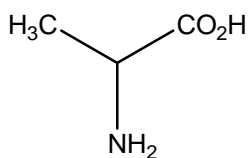
Use a glass capillary sealed from one side. Place the non-sealed end of the capillary into a product crystals, then turn it sealed end down and throw several times down through a glass tube. Check that the sealed side of the capillary is filled with the product. Apply the ready capillary to a melting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

Questions

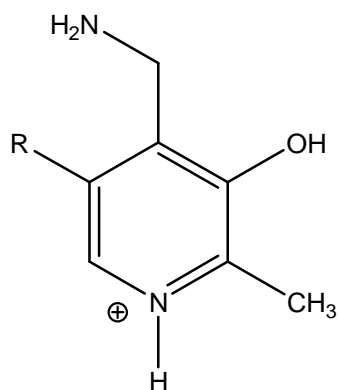
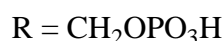
- 31.1** Draw the mechanism of imine formation. How can you name the intermediate?
What are the rate-limiting steps at low and high pH conditions?
- 31.2** What is similar and different in mechanisms of imine and acetal formation?
- 31.3** Draw the mechanism of vitamin B₆ derivative catalyzed transformation of pyruvic acid into alanine.



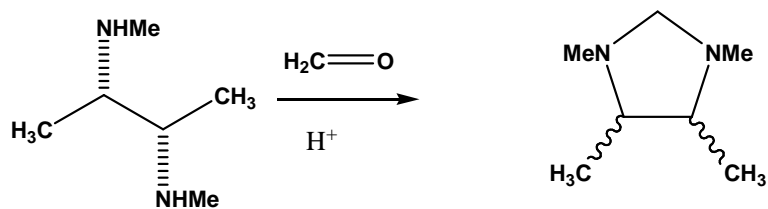
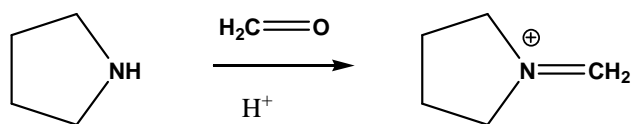
pyruvic acid

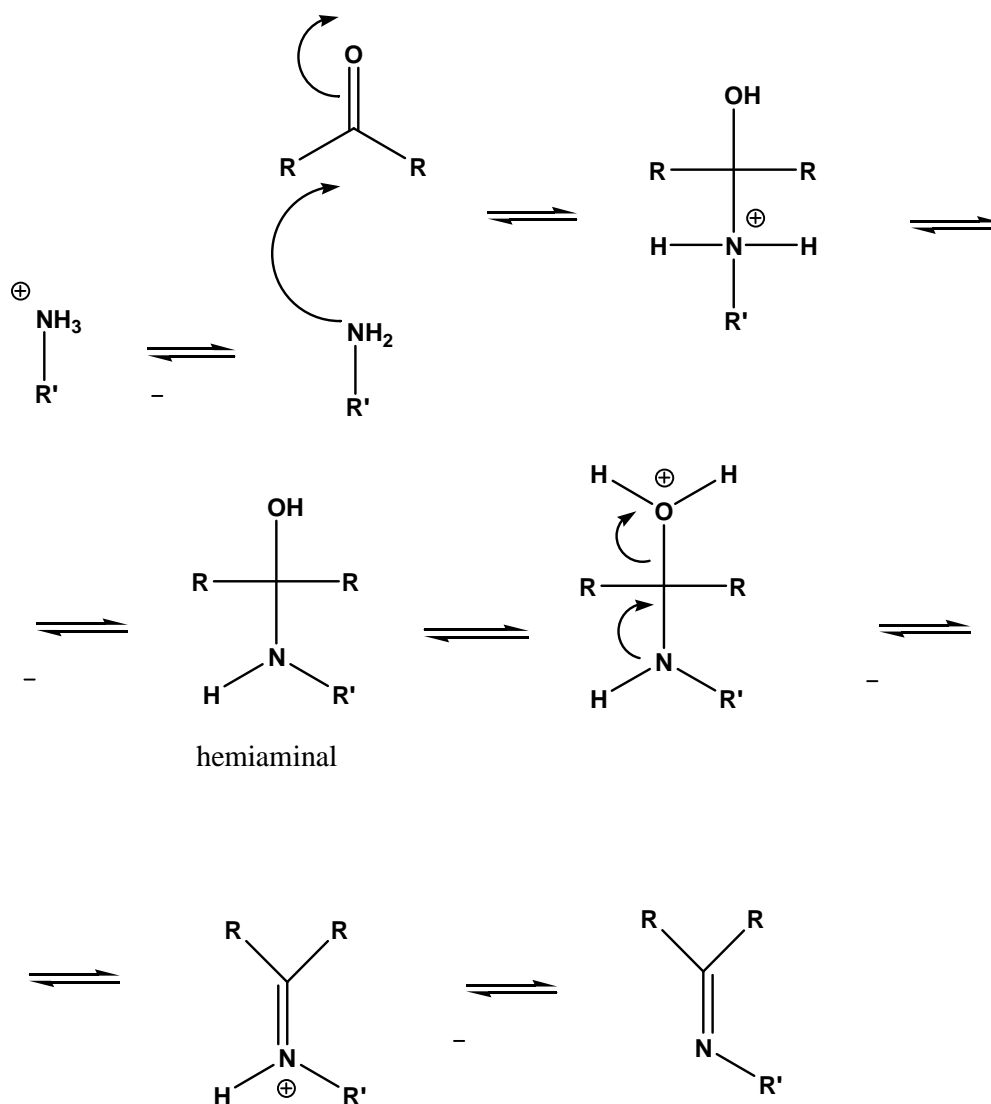


alanine

pyridoxamine phosphate,
⊖

- 31.4** Draw the mechanism of reductive amination of cyclohexanone into N,N-dimethyl cyclohexylamine using sodium cyanoborohydride and dimethylamine.
- 31.5** Suggest mechanisms for the two hereunder reactions. Draw the correct stereochemistry for the product of the second reaction.



SOLUTION OF PREPARATORY PROBLEM 31**Formation of double carbon-nitrogen bond****31.1 Hemiaminal.**

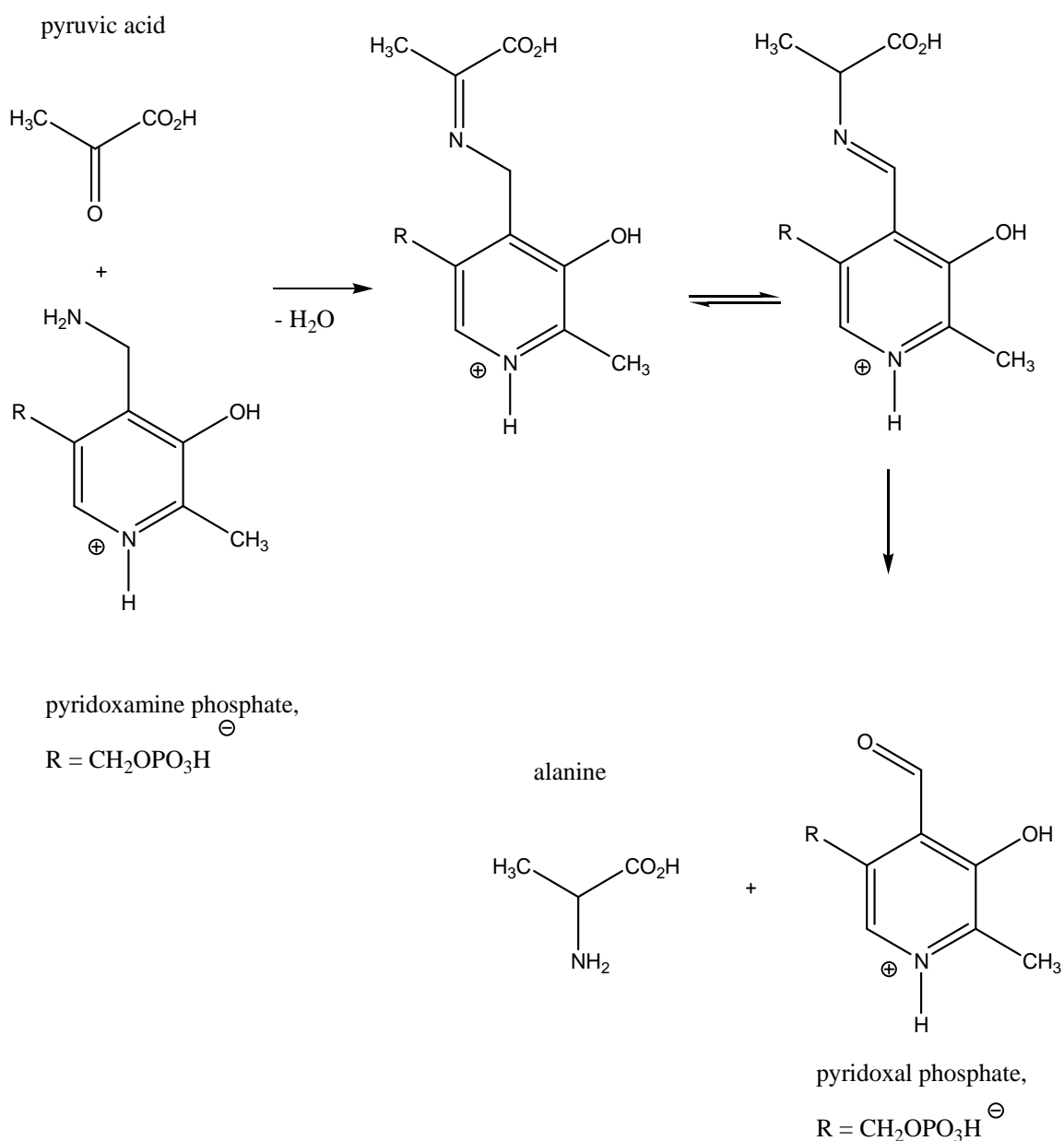
The rate-limiting steps are:

The amine attack at the carbonyl carbon atom at low pH, since most of the amine molecules are protonated;

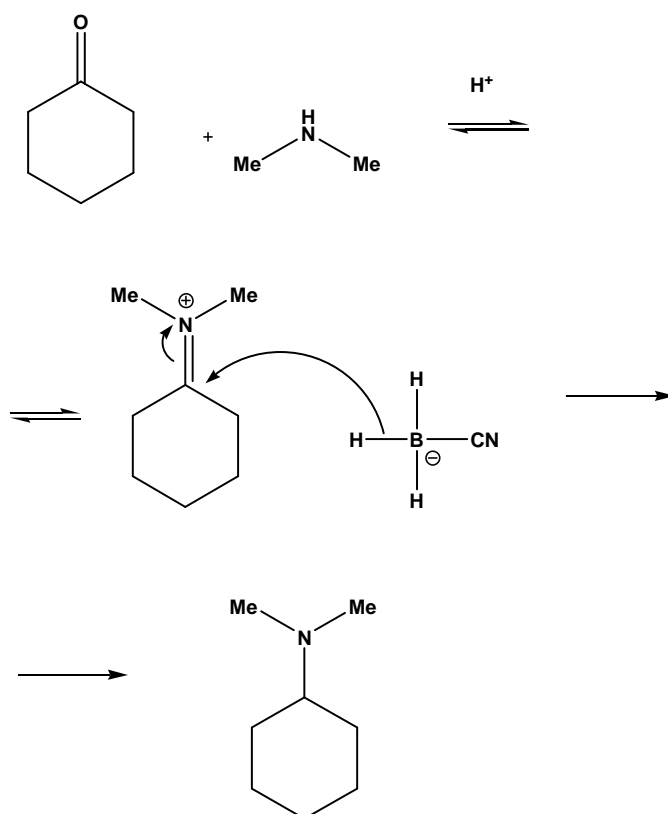
Dehydration of the tetrahedral hemiaminal intermediate at high pH, since this requires protons.

31.2 Both reactions proceed through the positively charged intermediates, iminium and oxonium ions, respectively. While the former just loses the proton to form the final product, the later acts as an electrophile adding another molecule of alcohol to become the full acetal.

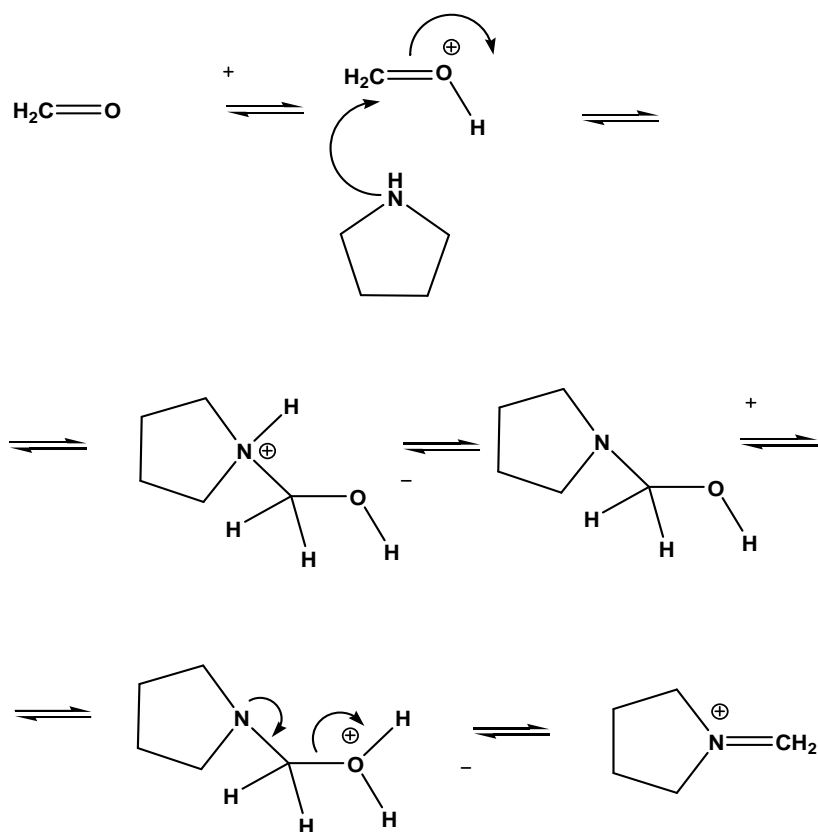
31.3

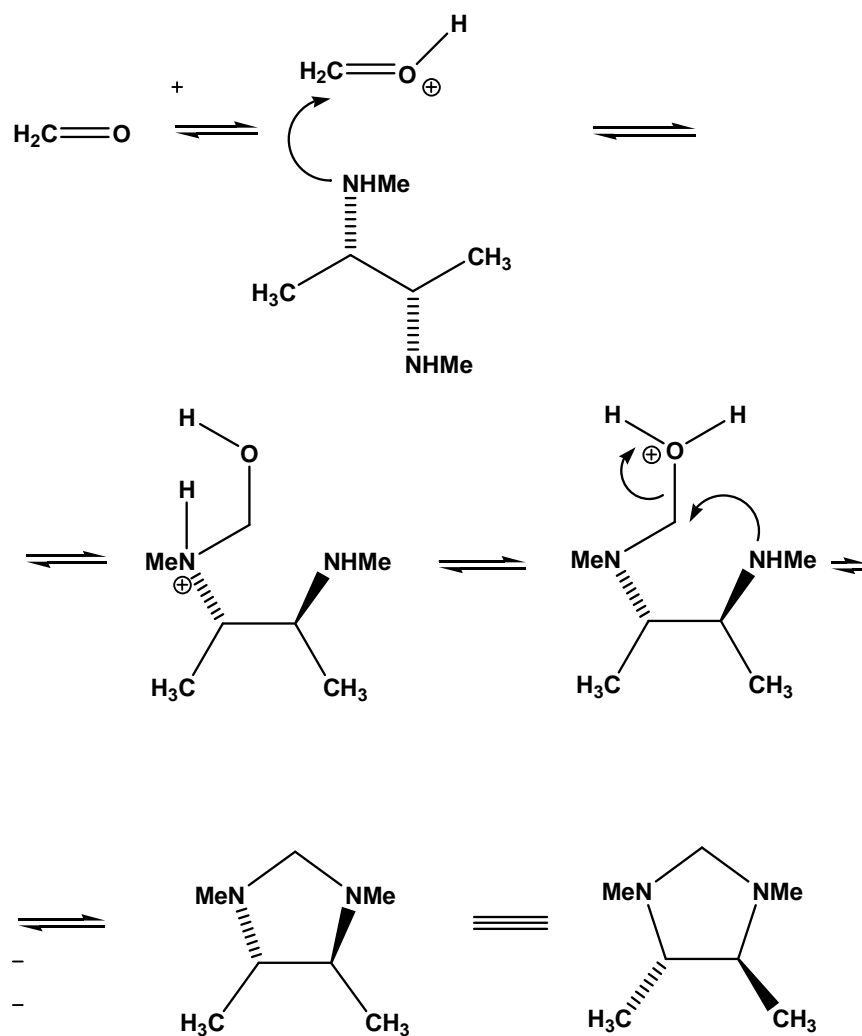


31.4



31.5

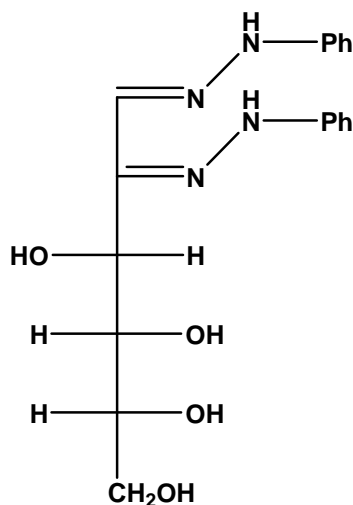




PREPARATORY PROBLEM 32 (PRACTICAL)

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 year 1880 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 %

Equipment and Glassware

- Magnetic stirrer with heating

- Magnetic bar
- Water bath
- Round-bottom flask, 50 cm³
- 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 cm³ of water, 400 mg of freshly distilled phenylhydrazine (caution – poisonous!) and 0.4 cm³ 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 cm³ 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

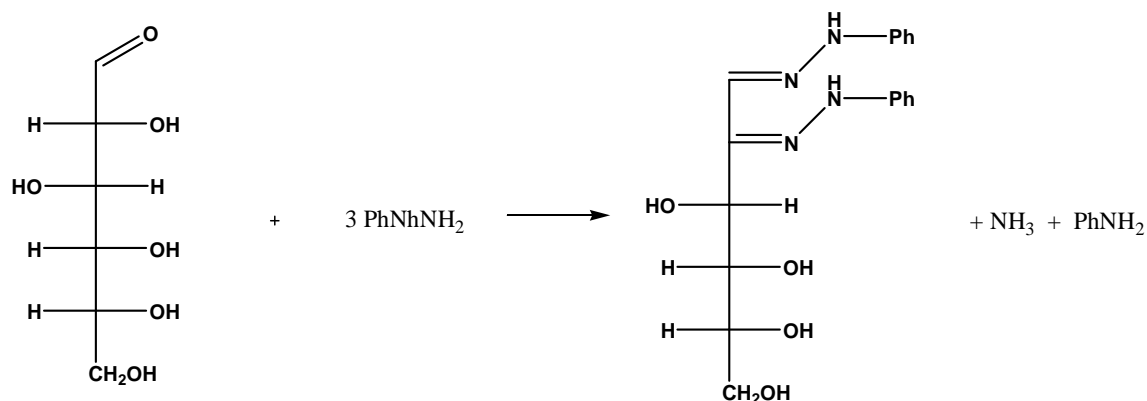
Determine the melting point of the product according to the directions in Problem 31.

Questions

- 32.1** Put the stoichiometry coefficients for the reaction between *D*-glucose and phenylhydrazine. What are the other products of this reaction?
- 32.2** Which starting substance would you use to calculate the yield of your product?
- 32.3** What is the product of the glucose reaction with equimolar amount of phenylhydrazine under mild conditions?
- 32.4** Draw the osazones of *D*-glucose, *D*-mannose and *D*-fructose. What can you say about the similarity in stereochemistry of the starting sugars?
- 32.5** 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
-

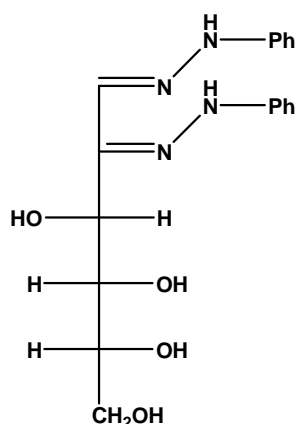
SOLUTION OF PREPARATORY PROBLEM 32

32.1

32.2 *D*-Glucose, since phenylhydrazine is taken in an excess.

32.3 The appropriate phenylhydrazone of aldehyde.

32.4



It is one and the same product for all the starting substances. This means the stereochemistry of C3, C4 and C5 of the starting sugars is the same. The initial difference in nature and/or stereochemistry at 1st and 2nd carbon atoms of the monosaccharides is equalized by hydrazone formation.

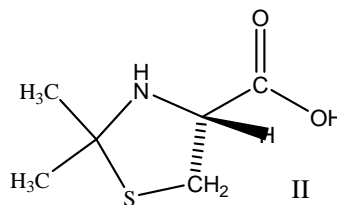
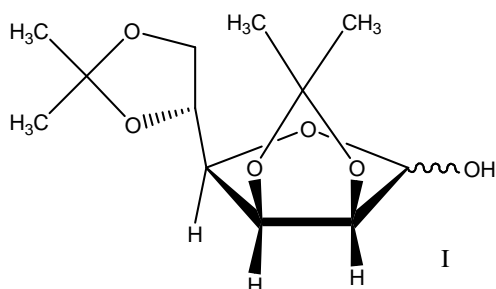
32.5 a), b), d) are different; c) are the same.

PREPARATORY PROBLEM 33 (PRACTICAL)

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).



Chemicals and Reagents

- *D*-Mannose, C₆H₁₂O₆,
- Iodine, crystalline
- Anhydrous acetone
- Na₂S₂O₃ solution, dilute
- Chloroform
- Na₂SO₄, calcined
- *L*-Cysteine hydrochloride
- Ninhydrine reagent (0.3 % sol-n of ninhydrine in n-butanol cont. 3% of sodium acetate)

- C₄H₉OH, n-butanol
- CH₃COONa, solution

Equipment and Glassware

- Magnetic stirrer with heating
- Magnetic bar
- Glass beaker, 50 or 100 cm³ (2 ea.)
- Round-bottom flask, 50 cm³
- 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 cm³ 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 Na₂S₂O₃ solution into the funnel and add it dropwise to the brown reaction mixture until the color disappearance. Add 10 cm³ 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 cm³ 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 cm³ of chloroform to the funnel and repeat the extraction procedure using the same beaker. Wash the combined organic layers with 10 cm³ of water using a clean separation funnel. Place calcined Na₂SO₄ 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 evaporator¹. 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-Cysteine with acetone

Fix a round-bottom flask on a stand. Place 100 mg of *L*-cysteine hydrochloride in 2 cm³ 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 glass filter off. Rinse the reaction flask with the

¹Can be done by a lab assistant. Students need not be trained in rotary evaporation.

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 cm³ 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

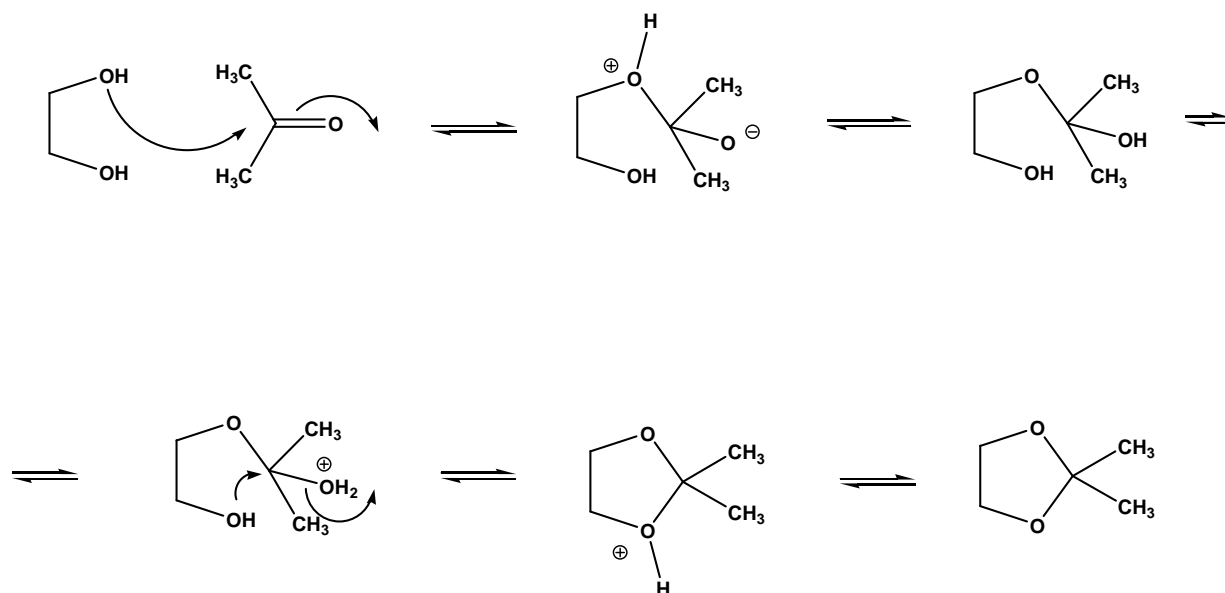
- 33.1** Draw the mechanism of formation of 1,3-dioxolane ring from acetone and 1,2-diol. Which catalyst acid or base, will you apply? Why?
- 33.2** Draw the products of acetone reaction with *trans*- and *cis*-cyclohexane-1,2-diols. Which of the products is thermodynamically more favorable?
- 33.3** 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?
- 33.4** What conditions and reagents would you apply to remove acetone protecting groups from diacetone mannose?

- 33.5** Draw the mechanism of product formation in the reaction of cysteine with acetone. Explain the role of hydrochloric acid.
- 33.6** 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.
-

SOLUTION OF PREPARATORY PROBLEM 33

Ninhydrine test. The spot with the product will show no color change, whilst that with the starting amino acid will become colored (blue-violet to brown-violet).

33.1



Transformation of hemiketal into full ketal needs the acid catalysis to protonate hydroxyl group, which is further removed in the form of water molecule. The resulting positively charged carbocation-type intermediate is stabilized by electron donation from oxygen lone pair.

33.2

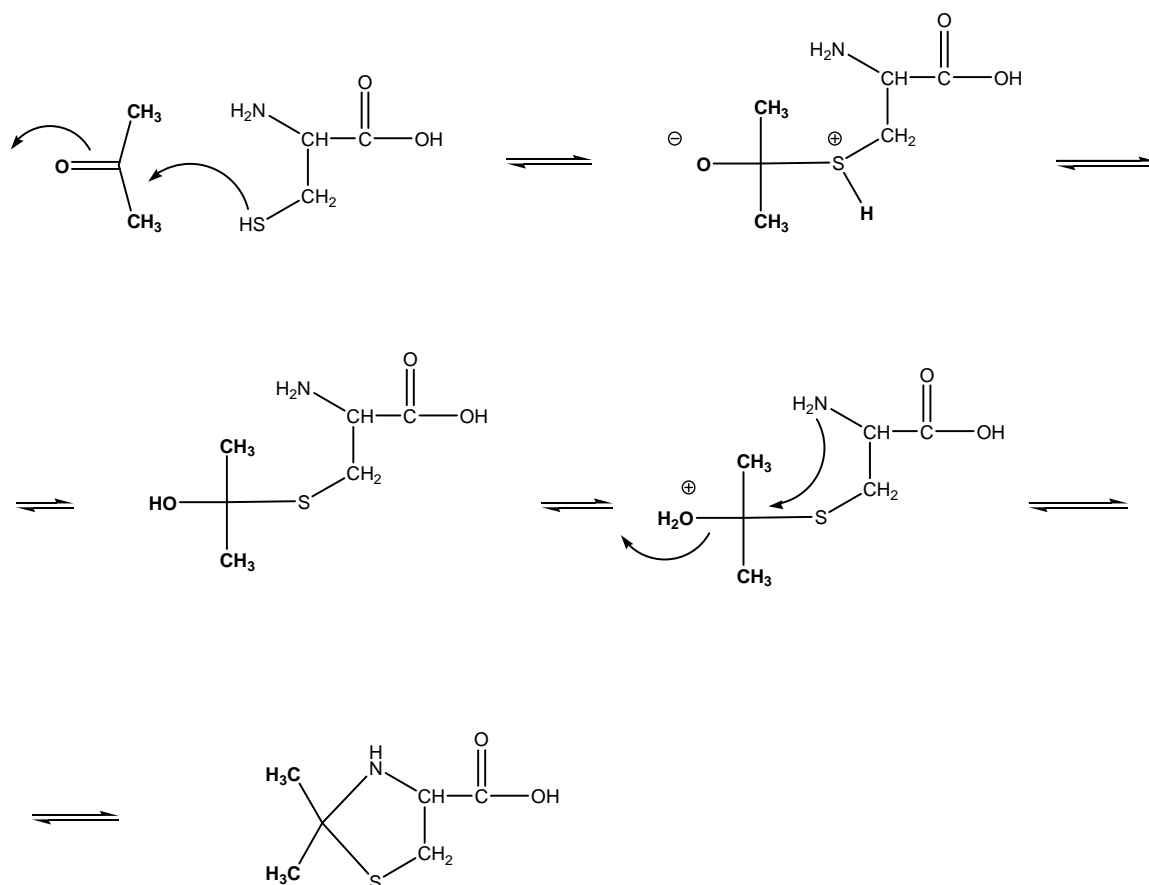


cis-Fused six- and five-membered rings in the resulting product of *cis*-cyclohexane-1,2-diol are more stable than *trans*-fused rings. The reason is the higher bond and angles distortion in *trans*-fused bicycles.

33.3 In the furanose form of *D*-mannose, there is a possibility to form two rather than one (in the pyranose form) 1,3-dioxolane rings, which is more thermodynamically favorable. Pyranose – furanose transformation proceeds via the open aldehyde form of the carbohydrate.

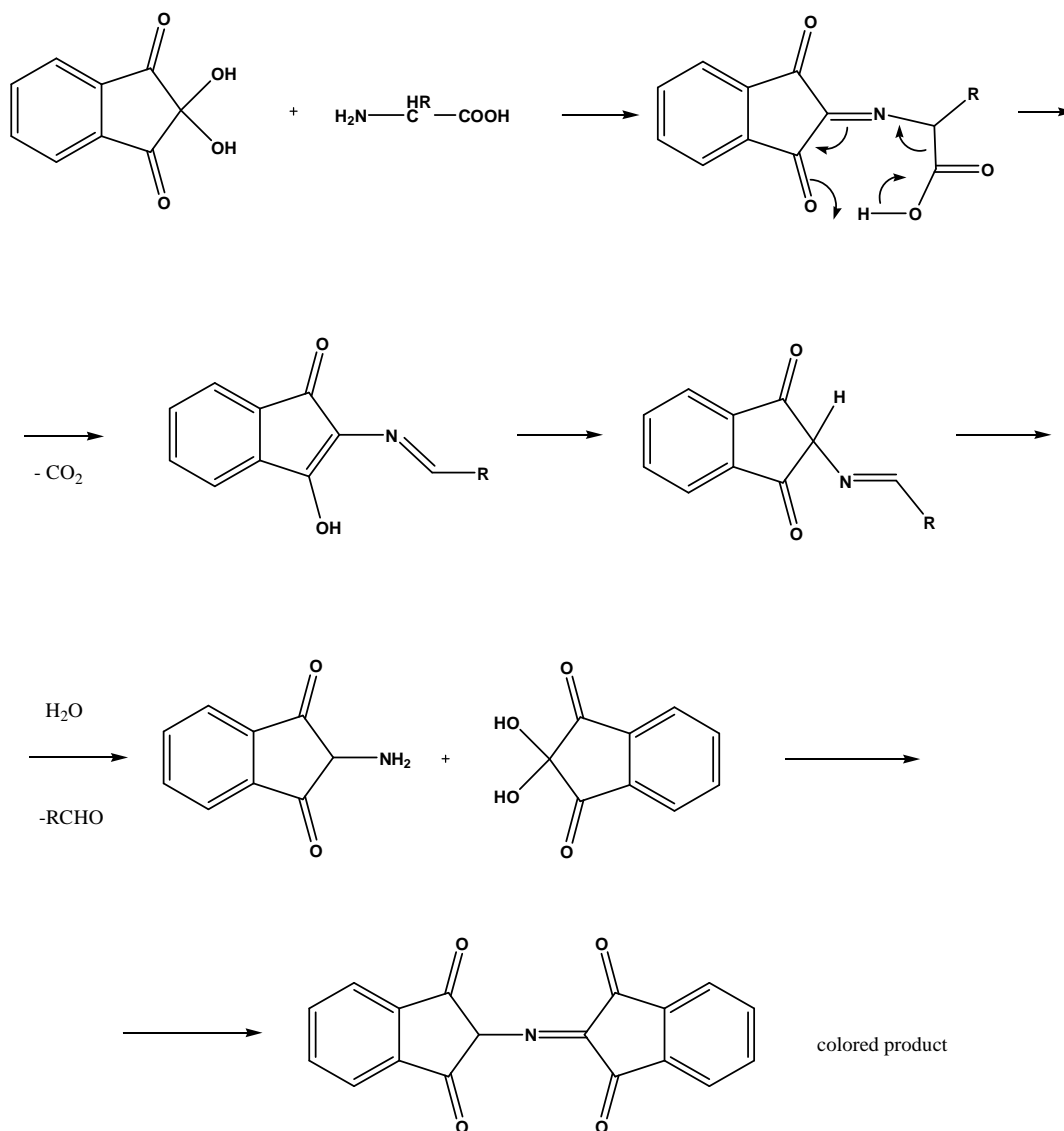
33.4 Aqueous hydrochloric acid.

33.5



Acid catalysis enhances the electrophilicity of carbonyl carbon atom (enhancing carbonyl activity). Thiol group reacts first due to higher nucleophilicity compared to that of amino group.

33.6



Problems 31-33. Melting points and yields of the products

Problem #	Product	Melting point, °C	Yield, %
31	<i>N</i> -[(<i>E</i>)-Phenylmethylene]aniline	51-53	85
32	Osazone of <i>D</i> -glucose	205-207	62
33	Acetone derivative of mannose	118-120	79
33	Acetone derivative of cysteine	148-150	68

PREPARATORY PROBLEM 34 (PRACTICAL)

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 $[\eta]$ 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 ($K = 0.017 \text{ cm}^3/\text{g}$, $a = 0.69$) and methyl ethyl ketone ($K = 0.039 \text{ cm}^3/\text{g}$, $a = 0.57$). All constants are given for 25 °C.

Chemicals and reagents

- Polystyrene (number-average molar mass of about 100 000) solution in toluene, 10 g cm^{-3} , 25 cm^3
- Polystyrene (number-average molar mass of about 100 000) solution in methyl ethyl ketone, 10 g cm^{-3} , 25 cm^3
- Toluene, 50 cm^3
- Methyl ethyl ketone, 50 cm^3

Apparatus and glassware

- Ubbelohde or other capillary viscometer
- Graduated cylinder, 10 cm^3
- 10 glass vials, 20 cm^3
- Volumetric pipette, 5 cm^3
- Stopwatch

Procedure

- For both polymer solutions, prepare a number of dilutions (in the concentrations range of 1 to 10 g/dm^3).
- Measure flow time for the solvent (toluene) using the Ubbelohde viscometer (repeat three times).
- Measure flow times for all polystyrene solutions in toluene (repeat each three times)
- Fill in the table below.
- Repeat ii. b) – d) for polystyrene solutions in methyl ethyl ketone.

Concentration c of the polymer (g dm^{-3})	Flow time t , s	$\eta_{\text{rel}} = \frac{t}{t_0}$	$\eta_{\text{sp}} = \frac{t - t_0}{t_0}$	$\frac{\eta_{\text{sp}}}{c}$, $\text{dm}^3 \text{g}^{-1}$

Questions and data analysis

- 34.1** Calculate the relative, specific and reduced viscosities for each solution studied
- 34.2** Plot the reduced viscosity against polystyrene concentration for each solvent.
- 34.3** Approximate the dependences from i. 2 with appropriate straight lines.
- 34.4** Determine the intrinsic viscosity of the polystyrene solutions in toluene and methyl ethyl ketone as Y-intercept.
- 34.5** Using the Mark-Kuhn-Houwink equation, determine the corresponding values of viscosity-average molar masses of the polystyrene sample.
- 34.6** Evaluate the polydispersity index of the polystyrene sample.

SOLUTION OF PREPARATORY PROBLEM 34

34.1 The viscosity values calculated from the flow times of polystyrene solutions (2 to 10 g/L) determined with the Ubbelohde viscometer at 25 °C are given in the hereunder tables. Each flow time value is an average of three measurements. Note that your experimental values may significantly differ from those in the tables, since the flow times depend on the molecular properties (mainly molecular weight distribution) of a particular polystyrene sample.

Polystyrene/toluene, the solvent flow time $t_0 = 24.4$ s

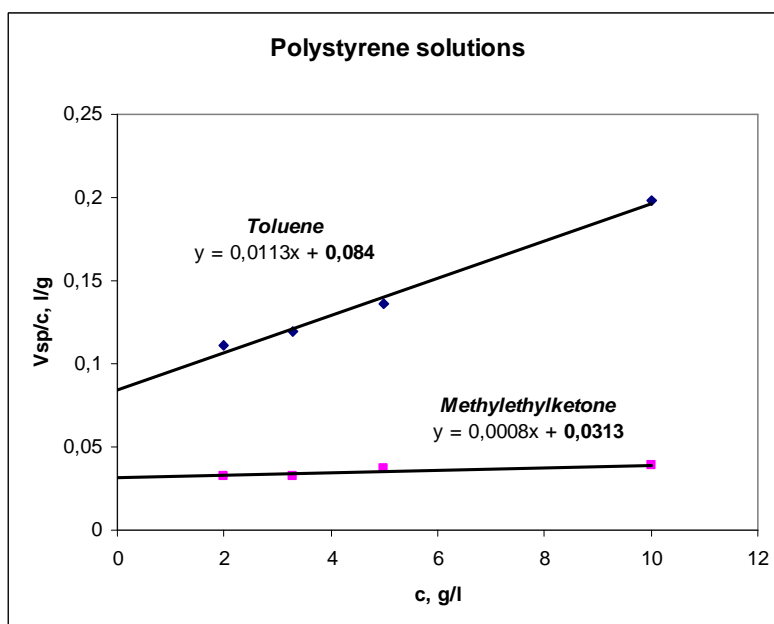
Concentration c of the polymer, (g dm ⁻³)	Flow time t , (s)	$\eta_{rel} = \frac{t}{t_0}$	$\eta_{sp} = \frac{t - t_0}{t_0}$	$\frac{\eta_{sp}}{c}$, (dm ⁻³ g ⁻¹)
10	72.8	2.98	1.98	0.198
5	41.0	1.68	0.68	0.136
3.3	34.0	1.39	0.39	0.119
2	29.8	1.22	0.22	0.111

Polystyrene/methyl ethyl ketone, the solvent flow time $t_0 = 26.0$ s

Concentration c of the polymer, (g dm ⁻³)	Flow time t , (s)	$\eta_{rel} = \frac{t}{t_0}$	$\eta_{sp} = \frac{t - t_0}{t_0}$	$\frac{\eta_{sp}}{c}$, (dm ³ g ⁻¹)
10	36.0	1.38	0.38	0.0385
5	30.8	1.18	0.18	0.0369
3.3	28.8	1.11	0.11	0.0326
2	27.7	1.07	0.07	0.0327

34.2 - 34.4

The intrinsic viscosity $[\eta]$ can be found by either graphical extrapolation to 0 concentration (as the Y-intercept), or by linear fitting (as an absolute term) of the reduced viscosity data.



Analysis of the data given in i. 1) leads to $[\eta]$ equal to 0.0840 and 0.0313 dm³ g⁻¹ for the toluene and methyl ethyl ketone solutions, respectively. (Three significant digits are left in both cases based on the typical amplitude of the measured flow times).

34.5 The viscosity-average molecular weights as calculated from the Mark-Kuhn-Houwink equation are of 226000 and 125000 g mol⁻¹ for the toluene and methyl ethyl ketone solutions, respectively.

34.6 The polydispersity index equals 226000 / 125000 = 1.81.

PREPARATORY PROBLEM 35 (PRACTICAL)

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

- $(C_4H_6O_2)_n$, poly(methacrylic acid (PMAA, molecular weight of 30000) aqueous solution, 2 g dm^{-3} , 50 cm^3
- $C_{2n}H_{4n+2}O_{n+1}$, poly(ethylene glycol) (PEG, molecular weights of 1000, 2000, 3000, 6000) aqueous solutions, 1 g dm^{-3} , 10 cm^3 of each solution
- Deionized water

Apparatus and glassware

- Ubbelohde viscometer or other capillary viscometer with thermostat
- Graduated cylinder, 10 cm^3
- 10 glass vials, 20 cm^3
- Volumetric pipette, 5 cm^3
- Stopwatch

Procedure

- Prepare a solution of PMAA with a concentration of 1 g dm^{-3} in water by diluting the initial solution of PMMA.
- 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).
- Measure the flow time of water at 25°C using the Ubbelohde viscometer (repeat three times)
- Measure the flow time of the prepared PMAA solution and of all mixtures at 25°C (repeat each three times).
- Fill in the table below.
- 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

- 35.1** Calculate the specific viscosity (see the explanation in Problem 34) for each of the measured samples.
- 35.2** Plot specific viscosity against molecular weight of PEG for each temperature.
- 35.3** Explain the dependences of the viscosity on temperature and molecular weight of PEG.

SOLUTION OF PREPARATORY PROBLEM 35

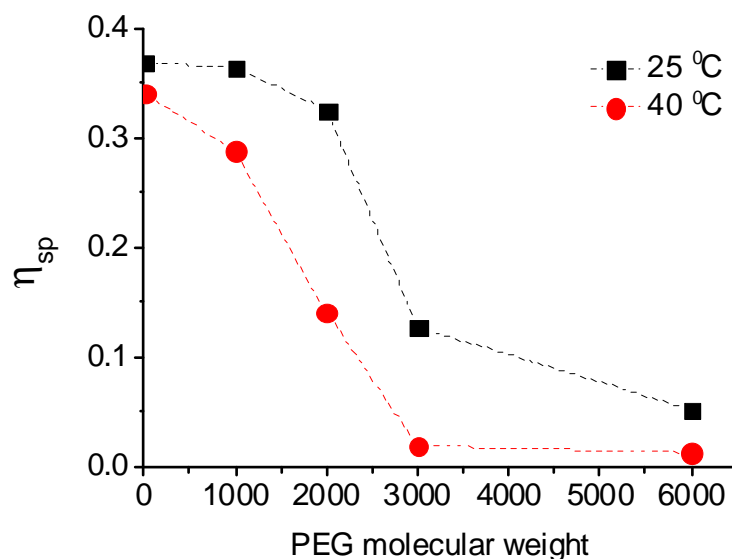
- 35.1** Experimental flow times and the calculated specific viscosities are given in the hereunder table.

Note 1. The molar masses of the repeating units of PMMA and PEG are of 86.06 and 44.05 g mol⁻¹, respectively. Mixing of equal volumes of a 2 g dm⁻³ PMMA and a 1 g dm⁻³ PEG (of any molecular mass) solutions results in a reaction mixture with the molar ratio of the PMMA and PEG units of approximately 1 : 1.

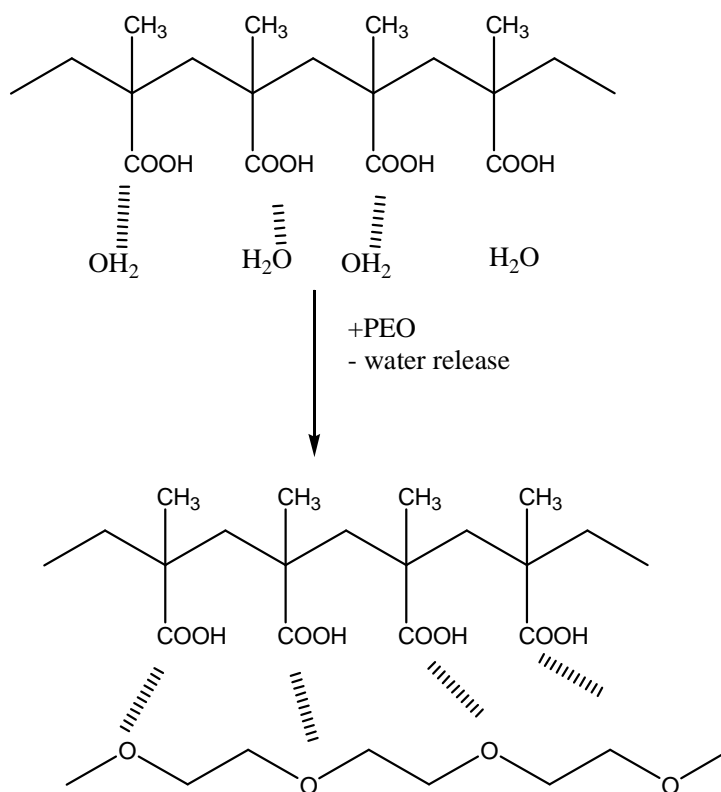
Note 2. The final concentration of PMMA in the resulting mixtures and its aqueous solutions is of 1 g dm^{-3} .

Composition	Temperature, °C	Flow time t , s	Specific viscosity of the solution η_{sp}
Water	25	44.0	-
PMAA, 1 g/L	25	60.2	0.368
PMAA+PEG-1000	25	60.0	0.364
PMAA+PEG-2000	25	58.3	0.325
PMAA+PEG-3000	25	49.6	0.127
PMAA+PEG-6000	25	46.3	0.052
Water	40	31.2	-
PMAA, 1 g/L	40	41.8	0.340
PMAA+PEG-1000	40	40.2	0.288
PMAA+PEG-2000	40	35.6	0.141
PMAA+PEG-3000	40	31.8	0.019
PMAA+PEG-6000	40	31.6	0.013

35.2



35.3 The reaction scheme of the complex formation is given below. A decrease of the specific viscosity of the PMAA solution upon addition of the equimolar amount of PEG is observed, which reflects that that polymer coils in the interpolymer complex are more compact than those in the initial solution. The compaction is due to hydrophobization of the PMAA chain with PEG.



Dramatic changes in the density of the complexes are observed within a rather narrow range of PEG molar masses (of about 1500 g mol^{-1} at 40°C and 2500 g mol^{-1} at 25°C). Such processes are often referred to as cooperative.

The enthalpy change in PMAA-PEG complex formation being negligible, the entropy gain due to the release of water molecules is the driving force of the reaction.

As positions of the repeating units in a polymer chains are constrained, the total entropy of the polymer coil is less than that of the same number of unbound monomer units. For longer polymer chains such entropy loss is more significant. Consequently, the entropy gain as a result of PMAA-PEG complex formation ($\Delta S = S(\text{complex}) + S(\text{water}) - S(\text{PMAA}) - S(\text{PEG})$) is increasing with an increase of the PEG chain length (total entropies of released water molecules, the complex, and the initial PMAA molecules are nearly the same). This is why the PMAA-PEG interaction proceeds efficiently only starting with a certain molar mass of PEG ($<1000 \text{ g mol}^{-1}$ at 40°C and of about $1000 - 2000 \text{ g mol}^{-1}$ at 25°C).

Higher efficiency of the complex formation at elevated temperatures (PEG with a lower molecular weight is needed to provide for a noticeable viscosity drop) contributes to the consideration that the entropy gain is behind the process.