Ministry of Health of the Republic of Belarus Educational institution ''Gomel State Medical University''

Department of General and Bioorganic Chemistry

Author: A.K. Dovnar, senior lecturer of the Department of General and Bioorganic Chemistry

METHODOLOGICAL MANUAL

for conducting the laboratory class with the first-year students of the Faculty of International Students studying in the specialty 7-07-0911-01 "Medical business" (FIS) English-speaking students in the discipline "Medical chemistry"

Topic 12: Chemical kinetics and catalysis

Time: 2 hours

THE TRAINING AND EDUCATIONAL GOALS, MOTIVATION TO STUDY THE TOPIC

The purpose of the class:

To acquaint students with the theoretical basis of the flow of chemical processes; to teach them to predict the influence of various factors on the change in the reaction rate and the shift of chemical equilibrium, as well as to form the skills of future doctors to experimentally determine the rate of chemical reactions.

The tasks of the class:

As a result of the class, the student must know:

1) basic concepts of chemical kinetics: rate and mechanism of a chemical reaction, reaction order and molecularity;

2) influence of various factors on the rate of chemical reaction;

3) kinetic equations of simple and complex reactions;

4) influence of temperature on the rate of chemical reactions;

5) the concept of catalysis and catalysts;

6) kinetic description of enzymatic reactions.

The student must be able to:

1) make kinetic equations of simple reactions;

2) calculate the half-life of radionuclides;

3) calculate the rate constants of chemical reactions of zero, first and second orders;

4) calculate the rate constants of chemical reactions of zero, first and second orders;

5) calculate the activation energy of chemical reactions based on kinetic data;

6) perform calculations using the Michaelis-Menten's enzymatic kinetics equation.

Motivation to study the topic:

Chemical kinetics is a branch of physical chemistry that studies the rate and mechanism of chemical reactions. The laws of chemical kinetics are widely used in the study of biochemical reactions occurring in the human body. One of the sections of pharmacology, called pharmacokinetics, studies the rate of absorption and the rate of excretion of drugs from the human body. The information obtained makes it possible to optimize the treatment process by increasing the effectiveness of the use of medicines. Pharmacokinetics is of great importance for medical science and clinical practice.

NECESSARY EQUIPMENT

1. Methodological manual for students on the topic "Chemical kinetics and catalysis".

2. Reference materials of physico-chemical constants for the 1st year education international students.

3. Chemical reagents and equipment necessary for laboratory work.

CONTROL QUESTIONS ON THE TOPIC OF THE CLASS

1. The subject of chemical kinetics. The rate and mechanism of chemical reactions. Kinetic curves. Factors affecting the reaction rate. Molecularity.

2. The Rate Law. Rate constant. Reaction order.

3. Kinetics of irreversible zero-order, first-order, and second-order reactions. The half-life of a reaction.

4. Kinetics of complex reactions: reversible, parallel, consecutive, conjugated, chain.

5. Temperature dependence of the reaction rate. Temperature coefficient. Activation energy. The Arrhenius' equation. Theory of the activated complex.

6. Catalysis and catalysts. Explanation of the action of the catalyst from the point of view of theory of the activated complex.

7. Kinetics of enzymatic reactions. The Michaelis-Menten's equation. Molecular activity of the enzyme (turnover number).

COURSE OF THE CLASS

The theoretical part

1. THE SUBJECT OF CHEMICAL KINETICS. THE RATE AND MECHANISM OF CHEMICAL REACTIONS. KINETIC CURVES. FACTORS AFFECTING THE REACTION RATE. MOLECULARITY

Chemical kinetics is the study of the rates of chemical reactions, the factors influencing these rates, and the reaction mechanisms by which reactions occur.

Chemical reactions, depending on the number and types of phases involved, can be divided into:

• homogeneous (occur in a single phase): $N_2(g) + 3H_2(g) \rightleftharpoons 2NH_3(g)$

• *heterogeneous* (reactants are components of two or more phases):

$$C(s) + O_2(g) \rightarrow CO_2(g)$$

The average rate of homogeneous reactions is defined as the change in the concentration of reactants or products per unit time:

$$v_{hom} = \pm \frac{[A] - [A]_0}{\tau}$$
, mol/l×s

[A]₀ and [A] – initial and final concentrations of a substance A, mol/l;

 τ – time of a reaction, s (min, hours);

(+) – a substance is produced;

(-) – a substance is consumed.

For example, for a chemical reaction: $2NO_2 \rightarrow 2NO + O_2$

the rate (v) of NO₂ consumption is equal to: $v = -\frac{[NO_2] - [NO_2]_0}{\tau}$;

the rate of O₂ production can be calculated as: $v = \frac{[O_2] - [O_2]_0}{\tau}$.

The kinetic curves of any simple chemical reaction are shown in Figure 1:

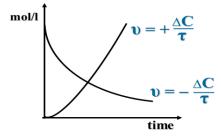


Figure 1 – The progress of a simple reaction

An average rate of heterogeneous reactions is defined as a change in moles of reactants or products per unit time per unit area of an interface surface:

$$v_{het} = \pm \frac{n(A) - n_0(A)}{\tau \times S}$$
, mol/m²×s

 $n_0(A)$ and n(A) – initial and final numbers of moles of a substance A, mol; S – an area of interface surface, m².

Factors affecting the reaction rate are:

- nature of reactants;
- their physical states;
- nature of solvents (for reactions in solutions);
- concentration of reactants (besides of zero-order reactions);
- pressure (for gaseous reagents);
- temperature;
- catalyst.

The reaction mechanism is a microscopic pathway by which reagents are converted into products. Each step is an elementary reaction. Species that are formed in one step and consumed in another are *intermediates*.

Each elementary reaction can be described in terms of its **molecularity**. The molecularity of an elementary reaction is the number of molecules that collide at this step:

- *unimolecular:* an elementary step involves one molecule: $I_2 \rightarrow 2I$;
- *bimolecular:* an elementary step involves two molecules: $H_2 + I_2 \rightarrow HI$;
- *termolecular:* an elementary step involves three molecules: $2NO + O_2 \rightarrow 2NO_2$.

Molecularity can't exceed three, since the probability of a collision of four particles is negligible.

According to the mechanism, the reaction can be:

• *simple:* the reaction mechanism involves only one elementary reaction:

 $H_2 + I_2 \rightarrow 2HI;$

• *complex:* the reaction mechanism includes several elementary reactions.

An example of a complex reaction is a chain or radical reaction, which results in the formation of free radicals as intermediates. Free radicals are particles with an unpaired electron. They are formed as a result of photolysis, thermolysis and redox reactions. The mechanism of chain reactions involves several types of steps (elementary reactions): initiation, propagation and termination.

Let's consider the reaction of methane with chlorine at elevated temperatures (400 °C–450 °C): $CH_4(g) + Cl_2(g) \rightarrow CH_3Cl(g) + HCl(g)$.

The mechanism of this chain reaction with the target product (CH₃Cl) in bold may be represented as follows:

1) initiation (unimolecular step):	$Cl_2 \rightarrow 2Cl$ ·			
(involves two bimolecular steps):	$Cl \cdot + CH_4 \rightarrow CH_3 \cdot + HCl$			
	$\mathrm{CH}_3{\boldsymbol{\cdot}} + \mathrm{Cl}_2 \longrightarrow \mathbf{CH}_3\mathbf{Cl} + \mathrm{Cl}{\boldsymbol{\cdot}}$			

3) <i>termination</i> (involves three bimolecular steps):	$CH_3 \cdot + Cl \cdot \rightarrow CH_3Cl$				
	$CH_3 \cdot + CH_3 \cdot \rightarrow H_3C - CH_3$				
	$Cl \cdot + Cl \cdot \rightarrow Cl_2$				

Radical reactions proceed *in vivo* under the effect of different free radicals OH, HOO, ROO, O₂ and some ones. Scientists came to the belief that accumulation of free radicals in intracellular fluids is one of the reasons for aging.

Rate of radical reactions can be increased greatly under the influence of radiation. Even small doses of radiation can accelerate generation of free radicals. Free radicals cause the destruction of cell membranes, suppress immune system of people, and cause different diseases. Radical reactions can be retarded by antioxidant substances: vitamins A, E, C and selenium compounds [1-3].

2. THE RATE LAW. RATE CONSTANT. REACTION ORDER

For a reaction to occur, the molecules of the reactants must collide. The rate of homogeneous reaction depends on a number of collisions of the reacting particles per unit of time. The number of collisions is directly proportional to the concentration of the reactants.

The Rate Law: the rate of a reaction is directly proportional to the reactants' concentrations (partial pressures of the reactants) raised to a powers.

For a hypothetical chemical reaction: $aA + bB + cC \rightarrow$ Products

the rate can be expressed as: $v = k \times [A]^x \times [B]^y \times [C]^z$

This equation is also known as the Rate Law.

k – rate constant which depends upon temperature and nature of substances and doesn't depend upon their concentrations; the units of the rate constant depend on the overall reaction order;

[A], [B], [C] – concentration of reactants, mol/l;

x, y and z – orders of a reaction by reactants (the orders with respect to A, B and C respectively);

n = x + y + z – the overall reaction order.

The order of a reaction can be determined experimentally. It may be positive or negative, integer or fractional, and zero as well.

Only for simple by mechanism reactions their orders coincide with coefficients in chemical equations [1-3].

3. KINETICS OF IRREVERSIBLE ZERO-ORDER, FIRST-ORDER, AND SECOND-ORDER REACTIONS. THE HALF-LIFE OF A REACTION

Zero-order reactions are the reactions for which rate of the reaction is independent of the reactant concentration.

Examples: photochemical, catalytic and enzymatic reactions with high concentration of a substrate.

= Zero-order reactions: n = 0

= The equation: $A \rightarrow P$

= The Rate Law:
$$v = -\frac{d[A]}{d\tau} = k \times [A]^0 = k$$

= The rate constant: $k = \frac{[A]_0 - [A]}{\tau}$, mol×l⁻¹×s

= *Half-life* $(\tau \frac{1}{2})$ is the time required for the concentration of a reactant to decrease by half of its initial concentration: $\tau \frac{1}{2} = [A]_0 / 2k$

= The kinetic curve:



Figure 2 – Zero-order reaction

First-order reactions are reactions in which the rate of the reaction depends only on the concentration of the reactant raised to the first power.

Examples: catalytic and enzymatic reactions *with low concentration of a substrate*: radio-active decay, elimination of drugs and their metabolites from a human body.

= First-order reactions: n = 1
= The equation:
$$A \rightarrow P$$

= The Rate Law: $v = -\frac{d[A]}{d\tau} = k \times [A]^1 = k \times [A]$
= The rate constant: $k = \frac{1}{\tau} \times \ln \frac{[A]_0}{[A]}$, s⁻¹
= Half-life: $\tau_{\frac{1}{2}} = \frac{\ln 2}{k} = \frac{0.693}{k}$
= The kinetic curve:
 $v = \frac{1}{2} \int_{k}^{\infty} \frac{1}{2$

Figure 3 – First-order reaction

Second-order reactions

Examples: hydrolysis of proteins, carbohydrates, lipids and other bioactive compounds.

= Second-order reactions: n = 2

= The equation:
$$2A \rightarrow P$$
 or $A + B \rightarrow P$
= The Rate Law: $\upsilon = k \times [A]^2$ or $\upsilon = k \times [A] \times [B]$
= The rate constant: $k = \frac{1}{\tau} \times \frac{[A]_0 - [A]}{[A]_0 \times [A]}, 1 \times \text{mol}^{-1} \times \text{s}^{-1}$
= Half-life: $\tau_{\frac{1}{2}} = \frac{1}{k \times [A]_0}$ or $\tau_{\frac{1}{2}} = \frac{1}{k \times [B]_0}$

Problem 1. In the treatment of some forms of cancer, a drug containing the radioisotope Ir-192 is used (its half-life is 74.08 days). Calculate how much Ir-192 will be contained in the human body through 10 days.

Solution:

$$\tau_{\frac{1}{2}} = \frac{\ln 2}{k} = \frac{0.693}{k}; \quad k = \frac{0.693}{74.08} = 9.35 \times 10^{-3} \, day^{-1}$$
$$k = \frac{1}{\tau} \times \ln \frac{[A]_0}{[A]}; \qquad \frac{[A]}{[A]_0} = e^{-k \times \tau} = e^{-0.00935 \times 10} = 0.91 \text{ or } 91\%$$

Answer: 91 %

4. KINETICS OF COMPLEX REACTIONS: REVERSIBLE, PARALLEL, CONSECUTIVE, CONJUGATED

Many reactions proceed by a mechanism that includes several elementary steps. They are called *complex reactions*. Among them are chain reactions, which we have already talked about. The kinetics of other types of complex reactions is summarized in Table 1.

Type of a reaction	Scheme	Rate Law					
Reversible reactions	$A \underset{k_2}{\overset{k_1}{\longleftrightarrow}} B$	$\upsilon = k_1 \times [A] - k_2 \times [B]$					
Parallel reactions	$\mathbf{A} \xrightarrow[k_2]{k_2} \mathbf{C}$	$\upsilon = k_1 \times ([A]_0 - [B]) + k_2 \times ([A]_0 - [C])$					
Consecutive reactions	$A \xrightarrow{k_1} B \xrightarrow{k_2} C$	The rate determining step governs the rate of the overall chemical reaction: $\upsilon = k_2 \times [B]$					

Table 1 – Kinetics of complex reactions

Conjugated reactions are those in which one proceeds only simultaneously with the other, i.e. it is induced by another reaction: a) $A + B \rightarrow M \iff b$ $A + C \rightarrow N$

For example, reaction (a) takes place only together with reaction (b), and substance C is the initiator of the first reaction. In biological systems, all endergonic reactions proceed according to the mechanism of conjugate reactions. An example of such a reaction in vivo is: hydrolysis of ATP (b) and synthesis of proteins and peptides (a) in a living organism. Hydrolysis of ATP ($\Delta G < 0$) is an exergonic reaction; formation of a peptide bond ($\Delta G > 0$) is an endergonic reaction. The Free Energy released during ATP hydrolysis is necessary for the synthesis of proteins and peptides in a living organism [2,3].

5. TEMPERATURE DEPENDENCE OF THE REACTION RATE. TEMPERATURE COEFFICIENT. ACTIVATION ENERGY. THE ARRHENIUS EQUATION. THEORY OF THE ACTIVATED COMPLEX

The most reactions obey the Van't Hoff's rule: for every 10° of temperature elevation, the rate of most reactions increases from 2 to 4 times.

$$\upsilon_{\mathrm{T}_{2}} = \upsilon_{\mathrm{T}_{1}} \times \gamma^{\frac{\mathrm{T}_{2} - \mathrm{T}_{1}}{10}}$$

 T_1 and T_2 – final and initial temperatures; γ – temperature coefficient of a chemical reaction; $2 < \gamma < 4$

The more accurate description of how reactions' rates depend upon temperature is given in the **Arrhenius' equation**:

$$\upsilon = \upsilon_0 \times e^{\frac{-E_a}{RT}}; \qquad k = k_0 \times e^{\frac{-E_a}{RT}}$$

 v_0 and k_0 – pre-exponential factors;

E_a – activation energy, kJ/mol.

The theory of activated complex defines *activation energy* as the minimum energy required for the reaction to occur. Activation energy is a barrier that prevents less energetic molecules from reacting.

According to the **theory of activated complex**, a pathway of a chemical reaction may be represented:

$\mathbf{A} + \mathbf{B} \rightleftarrows \mathbf{A} \dots \mathbf{B} \to \mathbf{A} \mathbf{B}$

activated complex

Before reactants turn into products an activated complex is generated. Activated complex is an energetically excited state that is intermediate between reactants and products. In activated complex the chemical bonds in reactants' molecules are not broken and new bonds are not formed.

The graphic representation of a pathways of endothermic and exothermic chemical reactions (Fig. 4):



Figure 4 – Energy diagrams of endothermic (a) and exothermic (b) reactions

Activation energy depends upon nature of reactants and doesn't depend upon temperature. Normally only a small fraction of the colliding molecules has enough kinetic energy to exceed the activation energy. Such particles are defined as *active molecules*. Increase in temperature gives increase in a fraction of active molecules contained in a mixture of reactants. Thus elevation of temperature results in accelerating of most chemical reactions [1-3].

6. CATALYSIS AND CATALYSTS. EXPLANATION OF THE ACTION OF THE CATALYST FROM THE POINT OF VIEW OF THEORY OF THE ACTIVATED COMPLEX

Catalyst is a substance that changes the rate of chemical reactions without itself being consumed. A reaction in which a catalyst is involved is a *catalyzed reaction*, and the process is called *catalysis*.

From the point of view of activated complex theory catalysts change the rate of chemical reactions through changing their pathways.

Without a catalyst: $A + B \rightleftharpoons A...B \rightarrow AB$ With a catalyst: $A + B + C \rightleftharpoons A...C...B \rightarrow AB + C.$ Catalysts may be:positive; negative.

Positive catalysts decrease activation energy of a reaction, increase a fraction of active molecules in a mixture of reactants at a constant temperature. As a result, the rate of reactions increases.

Negative catalysts increase activation energy of a reaction, decrease a fraction of active molecules in a mixture of reactants at a constant temperature. As a result, the rate of reactions decreases.

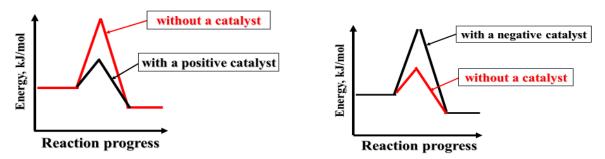


Figure 5 – The effect of positive and negative catalysts on the activation energy of exothermic reaction

An autocatalysis is a process when a product of a reaction acts as a catalyst. An example of an autocatalytic reaction (catalyst: Mn^{2+}) is:

 $2KMnO_4 + 5H_2C_2O_4 + 3H_2SO_4 \rightarrow 2MnSO_4 + 10CO_2 + K_2SO_4 + 8H_2O$

7. KINETICS OF ENZYMATIC REACTIONS. THE MICHAELIS-MENTEN EQUATION. MOLECULAR ACTIVITY OF THE ENZYME (TURNOVER NUMBER)

Enzymes are biological catalysts. Enzymes increase the rates of chemical reactions under physiological conditions: 37 °C and physiological pH. Most enzymes are proteins (although some ribonucleic acid molecules, termed ribosome, also have catalytic activity).

More than 2,000 enzymes were extracted from living systems; some of them (~150) are applied in medicine as drugs. Enzymes differ from other catalysts by:

a) very high catalytic activity (in some cases, enzymes can make a chemical reaction millions of times faster than it would have been without it);

b) high selectivity (one enzyme activates only one or two biochemical reactions).

German biochemist Michaelis and Canadian biochemist Menten proposed a mechanism to explain the dependence of the initial rate of enzyme-catalyzed reactions on concentration. The pathway of a single substrate enzymatic reaction:



S-a substrate, E- an enzyme, ES- enzyme-substrate complex, P-a product of a reaction.

The first step of an enzymatic reaction is characterized by a Michaelis' constant – K_M .

$$K_{M} = \frac{\left[S\right] \times \left[E\right]}{\left[ES\right]}$$

 K_M characterizes stability of enzyme-substrate complex (ES). The lower is K_M , the higher is stability of a complex.

The Rate Law of an enzymatic reaction: $\upsilon = k_2 \times [ES]$,

 k_2 is a first-order rate constant, known as **molecular enzyme activity** (turnover number), min⁻¹ is a number of substrate molecules converted into products under the effect of one enzyme molecule per unit time at 298 K. For most reactions k_2 values are in the range of $1 \times 10^4 < k_2 < 6 \times 10^6 \text{ min}^{-1}$.

A concentration of an enzyme-substrate complex [ES] can't be measured experimentally, but can be derived through another experimental data.

Michaelis-Menten's equation is fundamental equation of an enzymatic kinetics:

$$\upsilon = \frac{k_2 \times [E]_{overal} \times [S]}{K_M + [S]}$$

A multiplication $k_2 \times [E]_{overall}$ is constant and is defined as υ_{max} (maximum rate). Maximum rate corresponds to the zero-order enzymatic reaction.

$$\upsilon = \frac{\upsilon_{max} \times [S]}{K_M + [S]}$$

• At low substrate concentrations ([S] \ll K_M) equation becomes:

- $\upsilon = \frac{\upsilon_{max}}{K_M} \times [S]$ the first-order kinetics (linear portion of the plot).
- At high substrate concentrations ([S] >> K_M):

 $\upsilon = \upsilon_{max}$ – zero-order kinetics (horizontal portion of the plot).

• When $[S] = K_M$, $\upsilon = \upsilon_{max}/2$, so K_M equals the concentration of S when the rate is half its maximum value.

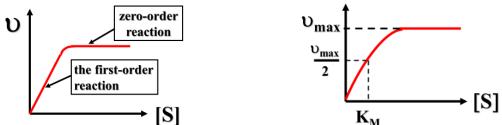


Figure 6 – Kinetic curves of enzymatic reaction

Kinetic curves of an enzymatic reaction we may use for graphical determination of υ_{max} and $K_{\text{M}}.$

The activity of enzymes is affected by:

a) temperature;

b) acidity of the medium;

c) the presence of inhibitors [2, 3].

The practical part

Safety instructions before laboratory work.

LABORATORY WORK

Effect of the reactant's concentration on the reaction rate

1. Chemical reactions that are used to determine the relationship between reactant's concentration and the rate of chemical reactions are:

 $\begin{array}{l} Na_2S_2O_3 + H_2SO_4 \rightarrow Na_2SO_4 + H_2S_2O_3 \\ H_2S_2O_3 \rightarrow H_2O + SO_2 + S \downarrow \end{array}$

The end of the reaction is determined by the appearance of turbidity of the solution, which is associated with the formation of a sulfur precipitate.

2. Fill 10 test tubes with a solution of sodium thiosulfate, sulfuric acid and water according to the scheme given in Table 1.

№ of a test tube	1	2	3	4	5	6	7	8	9	10
Solution of 1 M Na ₂ S ₂ O ₃ , ml	1		2		3		4		5	
Water, ml	4		3		2		1		0	
1 M H ₂ SO ₄ , ml		5		5		5		5		5
$Na_2S_2O_3$ concentration, mol/l	0.1		0.2		0.3		0.4		0.5	
Time when sulfur precipitate appears, s										

Table 1 – Dependence of reaction rate upon reactant's concentration

3. Mix $Na_2S_2O_3$ and H_2SO_4 solutions contained in test tubes 1 and 2, 3 and 4, 5 and 6, 7 and 8, 9 and 10, respectively. Note the time when the sulfur precipitate appears (the solution becomes turbid).

4. Calculate rates of a chemical reaction using formula: $v = 1/\tau$ and represent the obtained data in a graph form (a plot of rates against Na₂S₂O₃ concentrations).

5. Make a conclusion about the effect of the concentration of reacting substances on the rate of a chemical reactions.

Control over the assimilation of the topic

It is conducted in the form of an oral conversation with students.

QUESTIONS FOR SELF-CONTROL OF KNOWLEDGE

1. The rate and mechanism of chemical reactions. Kinetic curves. Factors affecting the reaction rate. Molecularity.

2. The Rate Law. Rate constant. Reaction order.

3. Kinetics of irreversible zero-order, first-order, and second-order reactions. The half-life of a reaction.

4. Kinetics of complex reactions: reversible, parallel, consecutive, conjugated, chain.

5. Temperature dependence of the reaction rate. Temperature coefficient. Activation energy. The Arrhenius' equation. Theory of the activated complex.

6. Catalysis and catalysts. Explanation of the action of the catalyst from the point of view of theory of the activated complex.

7. Kinetics of enzymatic reactions. The Michaelis-Menten's equation. Molecular activity of the enzyme (turnover number).

Exercises for the self – control

1. Indicate the Rate Law for the one-step reaction $A(s) + B(g) \rightarrow AB(s)$: a) $\upsilon = k \times [A]$; b) $\upsilon = k \times [A] \times [B]$; c) $\upsilon = k \times [B]$; d) $\upsilon = k$.

2. In the system: $CO(g) + Cl_2(g) \rightleftharpoons COCl_2(g)$, the concentration of CO was increased from 0.03 to 0.12 mol/l, and the concentration of chlorine was increased from 0.02 to 0.06 mol/l. How many times has the rate of direct reaction increased?

Answer: 12 times

3. Calculate the change in the reaction rate: $2NO(g) + O_2(g) \rightarrow 2NO_2(g)$ when:

a) the pressure was increased in 2 times;

b) the volume was decreased by 3 times.

Suppose that the reaction mechanism consists of one step.

Answer: a) will increase in 8 times; b) will increase in 27 times

4. The first-order reaction rate constant is $2.5 \times 10^{-5} c^{-1}$. How much of the substance will remain unreacted 10 hours after the start of the reaction? The initial concentration of the substance is 1 mol /l.

Answer: 0.407 mol/l

5. The temperature coefficient of the reaction is 2. How will the rate of chemical reaction change if:

a) the temperature was increased from 0 °C to 40 °C;

b) the temperature was lowered from 5 °C to -15 °C.

Answer: a) will increase in 16 times; b) will decrease in 4 times

QUESTIONS FOR INDEPENDENT WORK OF STUDENTS (IWS)

1. The mechanism of homogeneous and heterogeneous catalysis. Energy diagrams of the catalytic reactions.

2. Acid-base catalysis and its role in biological systems.

3. Enzymes as biological catalysts, features of their action. Factors affecting enzymatic reactions.

LIST OF SOURCES USED

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