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МОДЕЛЬНАЯ РЕАКЦИЯ РАЗЛОЖЕНИЯ ПЕРОКСИДА ВОДОРОДА ПОЛИМЕРМЕТАЛЛИЧЕСКИМИ КОМПЛЕКСАМИ

Аннотация

Освоение принципов функционирования живого обеспечивает возможность сознательного использования их в более простых молекулярно-организованных системах. Это и является предметом биомиметического подхода к решению многих химических задач. Если раньше под биомиметикой понимали «часть органической химии, пытающейся имитировать природные реакции и ферментативные процессы как средство увеличения возможностей органической химии», то в дальнейшем, это направление значительно расширилось.

Задачи биомиметической химии заключаются в моделировании биохимических процессов на молекулярном уровне и использовании результатов для получения «синтетических» ферментов («синзимов»), ферментоподобных систем, превосходящих по многим свойствам природные, создании искусственных переносчиков кислорода, синтетических материалов новой техники на основе биологических веществ, моделировании коферментов, ионофоров, фотопреобразующих устройств, проводящих материалов.

Ферменты и ферментоподобные системы, работающие по принципу металлоферментов и приближающиеся к ним по активности и избирательности действия, применяются в самых различных направлениях практической деятельности человека: в пищевой, фармацевтической, текстильной промышленности, в различных биотехнологических процессах, при создании ферментативных аналитических систем. К сожалению, широкое практическое применение нативных ферментов затруднено из-за их сложной технологии получения, связанной, прежде всего, с их лабильностью и экономической нецелесообразностью использования в гомогенных растворах. Эти недостатки можно устранить с помощью ферментов, связанных с носителем различными способами, т.е. путем иммобилизации. Вместе с тем малая доступность ферментов, особенно их чистых препаратов, лабильность и, вследствие этого ограниченность применения в экстремальных условиях создают предпосылки для создания более стабильных модельных биокаталитических систем, имитирующих активные центры различных ферментов. В связи с этим актуальным вопросом является создание новых модельных биокатализаторов на основе полимер-металлических комплексов.

Подобраны полимер-металлические катализаторы для разложения пероксида водорода. Изучено влияние полимерметаллических комплексов на скорость разложения пероксида водорода, а также что ионы железа связываются с полимерами при более низких концентрациях соли, по сравнению с другими переходными металлами и проявляют большую каталитическую активность в модельной реакции. Исследована устойчивость при хранении комплексов и их эффективность в реакции разложения пероксида водорода.

Ключевые слова: полимерметаллические комплексы, биокаталитическая активность, разложение пероксида водорода, гидрогели.

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СУТЕГІ АСҚЫН ТОТЫҒЫНЫҢ ПОЛИМЕРЛІ-МЕТАЛДЫҚ КОМПЛЕКСТЕРМЕН ЫДЫРАУЫНЫҢ МОДЕЛЬДІК РЕАКЦИЯСЫ

Аңдатпа

Тірі заттардың қызмет ету принциптерін меңгеру оларды қарапайым молекулалық ұйымдастырылған жүйелерде саналы түрде пайдалану мүмкіндігін береді. Бұл көптеген химиялық мәселелерді шешуге арналған биомиметикалық тәсілдің тақырыбы. Егер бұрын биомиметика «органикалық химияның мүмкіндіктерін арттыру құралы ретінде табиғи реакциялар мен ферменттік процестерге еліктеуге тырысатын органикалық химияның бір бөлігі» деп түсінілсе, кейінірек бұл бағыт айтарлықтай кеңейді.

Биомиметикалық химияның міндеттері – биохимиялық процестерді молекулалық деңгейде модельдеу және алынған нәтижелерді «синтетикалық» ферменттерді («синзимдер»), көптеген қасиеттері бойынша табиғидан жоғары ферменттәрізді жүйелерді алу, жасанды оттегі тасымалдаушыларын құру, биологиялық заттарға негізделген жаңа технологияның синтетикалық материалдары, коферменттерді модельдеу, ионофорлар, фотоконверттеу құрылғылары, өткізгіш материалдар.

Металлофермент принципі бойынша жұмыс істейтін және оларға белсенділік пен әсер ету селективтілігі бойынша жақындайтын ферменттер мен ферменттәрізді жүйелер адамның практикалық іс-әрекетінің әртүрлі салаларында қолданылады: тамақ, фармацевтика, тоқыма өнеркәсібінде, әртүрлі биотехнологиялық процестерде, ферментативті аналитикалық жүйелерді құру. Өкінішке орай, нативті ферменттерді кеңінен практикалық қолдану олардың күрделі өндіріс технологиясына байланысты қиын, бұл ең алдымен олардың лабильділігімен және біртекті ерітінділерде қолданудың экономикалық тұрғыдан тиімсіздігімен байланысты.

Бұл кемшіліктерді тасымалдаушымен әртүрлі тәсілдермен байланысқан ферменттерді қолдану арқылы жеңуге болады, мысалы: иммобилизация арқылы. Сонымен қатар, ферменттердің төмен қолжетімділігі, әсіресе олардың таза препараттары, лабильділігі және соның салдарынан экстремалды жағдайларда шектеулі пайдалану әртүрлі ферменттердің белсенді орталықтарын имитациялайтын анағұрлым тұрақты үлгідегі биокаталитикалық жүйелерді құруға алғышарттар жасайды. Осыған байланысты полимер-металл кешендері негізінде жаңа үлгідегі биокатализаторларды құру өзекті мәселе болып табылады.

Сутегі асқын тотығын ыдырату үшін полимер-металл катализаторлары таңдалды. Полимер-металл кешендерінің сутегі асқын тотығының ыдырау жылдамдығына әсері зерттелді, сонымен қатар темір иондары басқа өтпелі металдармен салыстырғанда төмен тұз концентрациясында полимерлермен байланысады және модельдік реакцияда үлкен каталитикалық белсенділік көрсетеді. Кешендерді сақтау кезіндегі тұрақтылық және олардың сутегі асқын тотығының ыдырау реакциясындағы тиімділігі зерттелді.

Түйін сөздер: полимер-металл кешендері, биокаталитикалық белсенділік, сутегі асқын тотығының ыдырауы, гидрогельдер.

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MODEL REACTION OF HYDROGEN PEROXIDE DECOMPOSITION BY POLYMETAL COMPLEXES

Abstract

Mastering the principles of functioning of living things provides the opportunity to consciously use them in simpler molecularly organized systems. This is the subject of the biomimetic approach to solving many chemical problems. If previously biomimetics was understood as “a part of organic chemistry that tries to imitate natural reactions and enzymatic processes as a means of increasing the capabilities of organic chemistry,” then later this direction has expanded significantly.

The tasks of biomimetic chemistry are to model biochemical processes at the molecular level and use the results to obtain “synthetic” enzymes (“synzymes”), enzyme-like systems that are superior to natural ones in many properties, the creation of artificial oxygen carriers, synthetic materials of new technology based on biological substances, modeling coenzymes, ionophores, photoconverting devices, conductive materials. Enzymes and enzyme-like systems, working on the principle of metalloenzymes and approaching them in activity and selectivity of action, are used in a variety of areas of practical human activity: in the food, pharmaceutical, textile industries, in various biotechnological processes, in the creation of enzymatic analytical systems. Unfortunately, the widespread practical use of native enzymes is difficult due to their complex production technology, which is associated primarily with their lability and economic impracticality for use in homogeneous solutions.

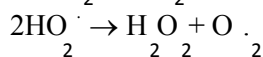
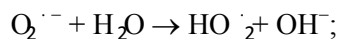
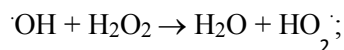
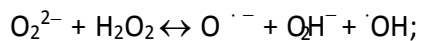
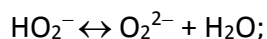
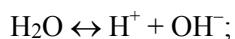
These disadvantages can be overcome by using enzymes bound to the carrier in various ways, e.g. by immobilization. At the same time, the low availability of enzymes, especially their pure preparations, lability and, as a result, limited use in extreme conditions, create the prerequisites for the creation of more stable model biocatalytic systems that imitate the active centers of various enzymes. In this regard, a pressing issue is the creation of new model biocatalysts based on polymer-metal complexes.

Polymer-metal catalysts have been selected for the decomposition of hydrogen peroxide. The influence of polymer-metallic complexes on the rate of decomposition of hydrogen peroxide has been studied, and also that iron ions bind to polymers at lower salt concentrations compared to other transition metals and exhibit greater catalytic activity in the model reaction. The stability during storage of the complexes and their effectiveness in the decomposition reaction of hydrogen peroxide were studied.

Keywords: polymer-metal complexes, biocatalytic activity, hydrogen peroxide decomposition, hydrogels.

Introduction. Enzyme modeling is one of the leading areas of research in the field of bioorganic chemistry. The role of enzymes in the living organism is very huge. Promising areas of bioorganic chemistry are the study of the structure of active centers, the mechanisms of operation of these centers, but also exclusively the catalytic properties of enzymes. In this regard, the need for research is the creation of enzyme-like systems that work on the principle of metalloenzymes and approach them in activity and selectivity of action. The main patterns of diverse redox catalytic reactions are easiest to establish by selecting and researching a suitable model. This model should have relative simplicity and, at the same time, universality of the course of redox reactions by a wide variety of mechanisms. Almost the only reaction that meets all these requirements is an oxidizer [1].

The decomposition of H₂O₂ is a useful model reaction for studying various redox processes, including enzymatic reactions. According to Kazarnovsky [2], homogeneous decomposition occurs in purified aqueous solutions of H₂O₂ through the following mechanism:



Transition metal ions or their complex compounds can serve as catalysts for model systems. It is well-established that transition metals are effective catalysts for redox processes, such as the decomposition of H_2O_2 . Polymer hydrogels with transition metal ions form complexes due to the presence of ionic macroligands and donor functional groups [3]. The formation of the complex occurs according to the ionic mechanism in the presence of negatively charged functional groups, according to the donor-acceptor mechanism with amino groups supplying a lone pair of electrons to the free d-orbitals of the metals. Researchers are showing great interest in studying such reactions, due to the specific structure of such complexes, which is reflected in the uniqueness of their properties. Experimental work in recent years has shown that metal ions play an essential role in a variety of biological processes. This is a significant finding that highlights the importance of metal ions in biological systems.

Materials and methods of research. The decomposition of hydrogen peroxide was carried out using polymer-metal complexes based on natural and synthetic polymers with transition metal ions. According to the method [5], the catalytic activity of these complexes was measured; the reaction was carried out in a 0.1 M phosphate buffer solution at pH = 7.0.

The following materials were used in the work: GPEI, GPAK, various salts of transition metals of chemically pure grade, without additional purification. Ferro- and potassium ferricyanide were also used, recrystallized according to the method [6]. It

is important to note that when working with acidic solutions of $\text{K}_4[\text{Fe}(\text{CN})_6]$, it is necessary to work under traction and wear rubber gloves. To dissolve 110 g of $\text{K}_4[\text{Fe}(\text{CN})_6] \cdot 3\text{H}_2\text{O}$ (technical), add 150 ml of water. The solution was evaporated until its density reached 1.2. Then, 0.1 g of activated carbon was added, stirred, and left to settle for 10 minutes. Afterward, the crystals that formed during the cooling of the filtrate were collected using a Buchner funnel, washed with 5 ml of water, and dried at room temperature. The yield was 70-73 g (approximately 65%).

Recrystallized technical salt was used to obtain Salt $\text{K}_3[\text{Fe}(\text{CN})_6]$. To do this, 130-135 g of $\text{K}_3[\text{Fe}(\text{CN})_6]$ (tech.) was dissolved in 350 ml of water with stirring and heating no higher than 70°C . The solution was then filtered and cooled, and the resulting crystals were collected on a

Buchner funnel, washed with a small amount of water, and dried at room temperature. The yield was 50 g (37-38%). The yield can be increased to 80-90% by repeatedly recrystallizing the salt using masterbatch solutions.

Polyacrylic acid gel (GPAK) was synthesized by radical polymerization of acrylic acid monomers in the presence of N,N-methylene bisacrylamide in an aqueous medium at a temperature of 343 K. The redox system ($\text{Na}_2\text{S}_2\text{O}_3$ and $\text{K}_2\text{S}_2\text{O}_8$) was used as the initiator [7].

Polyethylenimine gel (GPEI) was synthesized following the method described in [8]. A 10% solution of a previously obtained polymer in a dimethylformamide medium was used in the presence of an epoxy crosslinking agent, epichlorohydrin, at a temperature of 338 K until gelation occurred. The resulting cross-linked polymer was treated with a 0.1 M hydrochloric acid solution and repeatedly washed with a large volume of water for two weeks.

Interpenetrating polymer meshes based on the natural polymer agar-agar and synthetic polymers —

polyacrylic acid, polyacrylamide, polyethylenimine, and hydrogels based on PAA with different contents of sodium humate, synthesised in the physical chemistry laboratory, were also used.

Chemically pure (C.P.) grade sodium phosphates were used to prepare the buffer solutions. The composition of the 0.1 M buffer solution at different pH values is given in Table 1. 0.025 n KMnO₄ solution was prepared from fixanal (0.1 g -eq/l). Sulphuric acid, hydrochloric acid, phosphoric acid, sodium chloride and hydrogen peroxide were as specified in “C.P.”

Table 1 – Composition of 0.1M phosphate buffer system

pH	0,1M Na ₂ HPO ₄ (ml)	0,1M NaH ₂ PO ₄ (ml)	[Na ⁺]
1	2	3	4
4,75	0,0	100,0	0,1000
5,80	8,0	92,0	0,1080
6,0	12,3	87,7	0,1123
6,20	18,5	81,5	0,1185
6,40	26,5	73,5	0,1265
6,60	37,5	62,5	0,1375
6,80	49,0	51,0	0,1490
7,00	61,0	39,0	0,1610
7,20	72,0	28,0	0,1720
7,40	81,0	19,0	0,1810
4,60	87,0	13,0	0,1870
7,80	91,5	8,5	0,1915
8,00	94,7	5,3	0,1947
9,45	100,0	0	0,2000

Complexes of polymer hydrogels with transition metal ions were prepared by mixing hydrogels pre-swollen in water with solutions of salts of various concentrations. Gel samples of equal weight (0.01 g) were filled with 10 ml of distilled water and kept for one day. Solutions of transition metal salts of a certain concentration were then added to the swollen gel samples and allowed to stand for 2-4 hours before the system reached equilibrium.

The results and their discussion.

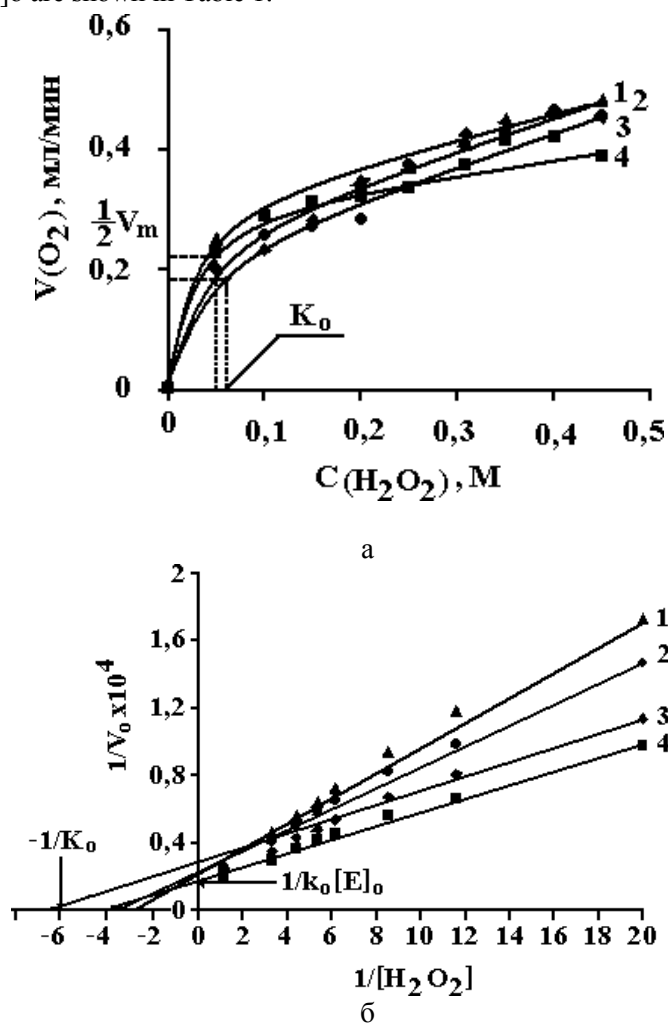
To study the effect of solution pH on the rate of hydrogen peroxide decomposition, 20 mg of the complex and 10 ml of 0.1 M phosphate buffer solution with different pH values from 3.5 to 9.0 were added to a thermostated cell. Catalase activity was determined after 3 min. [9]

As a result of studying the dependence of catalase activity on the pH of the medium, a bell-shaped dependence was obtained. This dependence is due to the following processes. Change in the concentration of catalytically active centers due to the rearrangement of gel/Men⁺ complexes, as well as the dissociation of hydrogen peroxide molecules (pK_a = 11.6). The catalase activity of polymer complexes is maximum at pH = 6.5-7.5, i.e. in the region where the functional groups involved in complex formation are deprotonated. The observed decrease in the activity of the complex in the acidic and alkaline regions is associated with pH-dependent conformational transitions associated with the ionization of functional groups. The pH stability of the resulting polymer-metal complexes corresponds to the pH stability of the soluble form of the enzyme (5.0 to 7.0).

The rate of decomposition of hydrogen peroxide by polymer catalysts has been studied. The Michaelis type curves shown in Figures 1a, 2a, 3a are obtained, which are straightened in Lineweaver-Burke coordinates (Figures 1b, 2b, 3b).

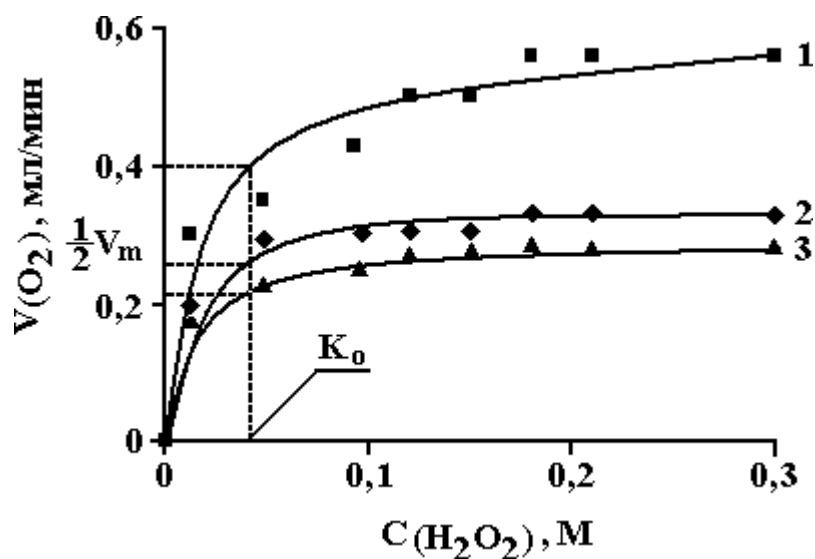
After presenting the results of the dependence of V_o on [S]_o on a graph with coordinates 1/V_o and 1/S_o, we extended the obtained experimental line to the intersection with the ordinate axis and then the abscissa. This allowed us to find the values of 1/C_o along the segments cut off on the axes [E]_o and -1/C_o. The values of the

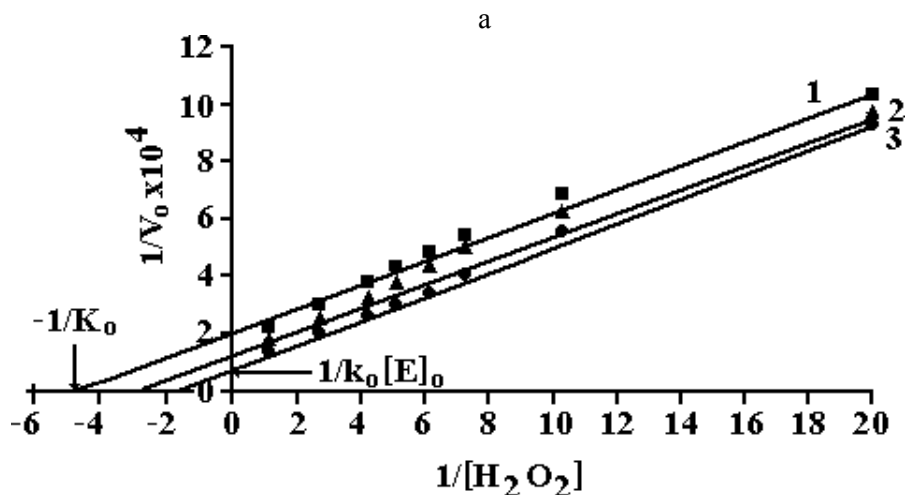
constants $1/Co$ and $1/Co[E]_0$ are shown in Table 1.



1- ГПЭИ-FeCl₃, 2-ГПАК-FeCl₃, 3-ГПЭИ-K₃Fe(CN)₆, 4- ГПЭИ-K₄Fe(CN)₆ [Fe³⁺]=1x10⁻⁵М, [K₄Fe(CN)₆]=1x10⁻²М, [K₃Fe(CN)₆]=1x10⁻²М, pH 7,0, T=298 К

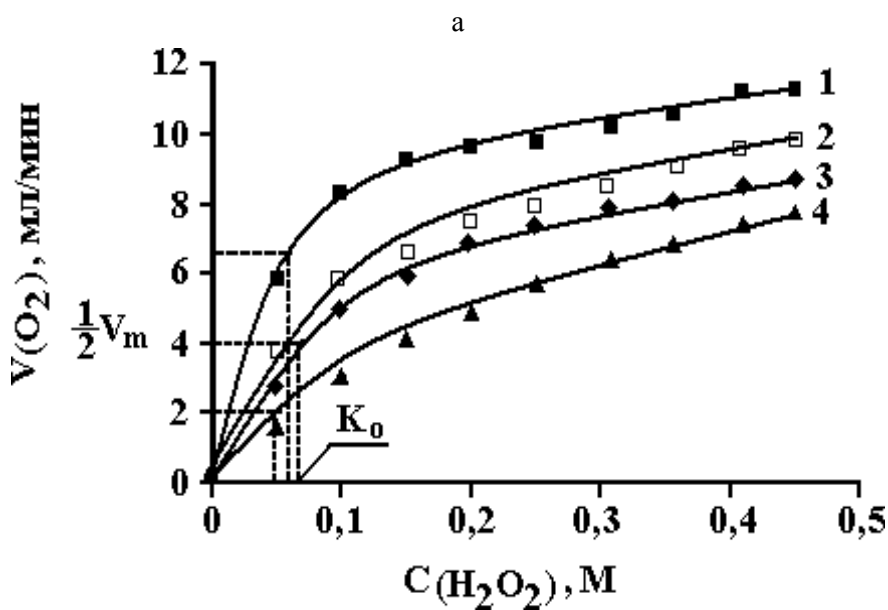
Figure 1- Dependence of the rate of decomposition of hydrogen peroxide on the concentration of PV (a) and the dependence of $1/V_0$ on $1/[PV]$ (b)

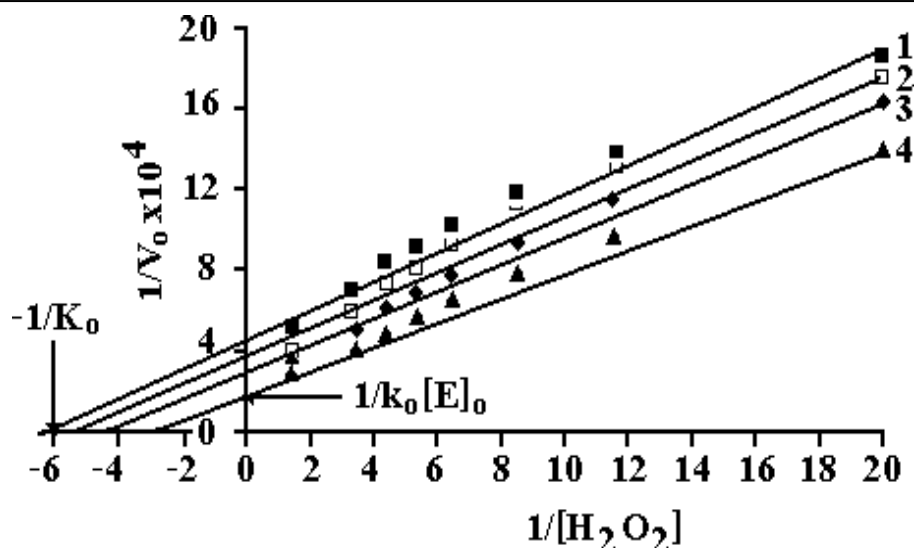




1-Аг-Аг-ПЭИ+ FeCl₃, 2-Аг-Аг-ПАК+FeCl₃, 3-ГПАА-ГNa+ FeCl₃ [Fe³⁺]= 1x10⁻⁵М, рН 7,0, Т = 298 К

Figure 2 - shows the relationship between the reaction rate and the concentration of PV (a), as well as the relationship between $1/V_0$ and $1/[PV]$ (b).





б

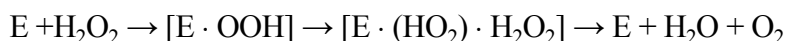
- 1- FeCl₃-ГПАК-ЭДА, [Fe³⁺]=1x10⁻⁵М, [ЭДА]=1x10⁻¹М, pH 10,4, T = 298 К
- 2- FeCl₃-П-Аг-Аг-ПЭИ, [Fe³⁺]=5x10⁻²М, [PP]=1x10⁻¹М, pH 7,0, T=298 К
- 3- FeCl₃-ГПЭИ-ДБСNa, [Fe³⁺]=5x10⁻¹М, [ДБСNa]=3x10⁻²М, pH 7,0, T=298 К
- 4- FeCl₃-ПАК-ГПЭИ, [Fe³⁺]=5x10⁻⁵М, [ПАК] = 1x10⁻³М, pH 7,0, T = 298 К

Figure 3 - shows the relationship between the reaction rate and the concentration of PV (a), as well as the relationship between 1/Vo and 1/[PV] (b).

Table 2 – Constants 1/Co and 1/Co[E]o 1/Ko and 1/Ko[E]o

Catalysts	1/Co, М	1/Ko[E]o, с ⁻¹
ГПЭИ-К4Fe(CN) ₆	0,25	3,3x10 ⁴
ГПЭИ-К3Fe(CN) ₆	0,16	3,3x10 ⁴
ГПЭИ-FeCl ₃	0,4	4,0x10 ⁴
ГПАК- FeCl ₃	0,26	4,0x10 ⁴
Аг-Аг-ПАК-FeCl ₃	0,35	0,7x10 ⁴
Аг-Аг-ПЭИ-FeCl ₃	0,65	0,5x10 ⁴
ГПАА-ГNa-FeCl ₃	0,55	1,0x10 ⁴
FeCl ₃ -ПАК-ГПЭИ	0,36	0,25x10 ⁴
FeCl ₃ -ГПАК-ЭДА	0,16	0,24x10 ⁴
FeCl ₃ -ГПЭИ-ДБСNa	0,25	0,33x10 ⁴
FeCl ₃ -П-Аг-Аг-ПЭИ	0,19	0,26x10 ⁴
Catalaze (-α form)	1,1	7x10 ⁷
Catalaze (-β form)	1,1	3x10 ⁶

The rate constant for the decomposition of hydrogen peroxide by polymer-metal catalysts is significantly lower compared to catalase. Despite this research result, model polymer-metal complexes exhibit sufficient catalase activity, which proves the possibility of using these systems as biocatalysts. It should be noted that the resulting complexes are stable during long-term storage, and the native enzyme loses activity very quickly. It is possible that the mechanism of activation of the decomposition of hydrogen peroxide in the studied complexes is similar to the mechanism of the catalase reaction according to the scheme [10]:



Thus, some features are characteristic of model polymer catalysts, including properties such as the pre-activation of hydrogen peroxide molecules in the reaction zone, and possibly the interaction between the active centres themselves. Of all the systems studied, the FeCl₃-Ag-Ag- PEI and CuCl₂-Ag-Ag-PEI systems have the highest catalase activity and stability. The difference between the enzyme catalase and its models is explained by the different spatial structure of the molecule.

Conclusion. The main polymer-metal complexes that can be used as models of biocatalysts have been selected. Complexes based on polymer hydrogels with transition metal ions, as well as semi-interpenetrating networks with transition metal ions, which exhibit catalase activity during the decomposition of hydrogen peroxide, have been obtained. The formation and activity of complexes depends on the nature of both the metal and the polymer. The presence of a natural polymer ensures “tuning” of the catalyst to the substrate.

As a result of studying the reaction rate of hydrogen peroxide decomposition by polymer-metal complexes, it was revealed that iron ions bind to polymers at lower salt concentrations compared to other transition metals and exhibit greater catalytic activity in the hydrogen peroxide decomposition reaction. To achieve maximum activity of the complexes in the decomposition of hydrogen peroxide, the following conditions are necessary: neutral pH of the solution, low salt concentration in the complex, the presence of an amine-containing component, a natural polymer, and the concentration of hydrogen peroxide.

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