

BIOCATALYTIC ACTIVITY OF VARIOUS BIOPOLYMERIC COMPLEXES WITH TRANSIENT METAL IONS

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ABSTRACT

In the recent years more and more attention is paid to the problems of molecular engineering and biomimetics. Complexes of transitional metals with polymer hydrogels and interpenetrating polymer networks manifesting high biocatalytic activity are of a special interest. These catalysts can be considered as closest analogs of metalloenzymes.

In this connection the study is focused on the following polymer-metallic systems: hydrogel of polyethyleneimine PIH–K₄Fe(CN)₆; PIH–K₃Fe(CN)₆; PIH–Cu²⁺-DBCNa interpenetrating nets on the basis of agar-agar and polyethyleneimine (Ag-Ag-PI) – Fe³⁺-phaeophytin (Php). The matrix swelling and adsorption ability as well as the binding degree of IPN with Fe-Php are reported. The IPN matrix dependence on porphyrin concentration is followed. The catalase activity of the complexes in the reaction of hydrogen peroxide decomposition is determined by the permanganometric method. The reaction is carried out in 0,1M phosphatic buffer solution at pH 7,0. The picture observed in case of polymer hydrogels with potassium ferrocyanates is also found valid for the polymer nets. The activities of the complexes obtained are found determined by the presence of a cationic polymer and the polymer net mobility. Thus, it is shown that the catalase activity is influenced by the quantitative and qualitative composition of the complex.

Keywords: hydrogel, interpenetrating networks, biocatalytic activity, ternary complexes, biopolymeric complexes, porphyrin, photocatalysts, complexformation and swelling degree.

INTRODUCTION

Immobilization technology has traditionally been focused on some main areas of interest like biomedical science and biotechnology. A large number of bioactive materials such as drugs, proteins, plant and animal cells, microorganisms of various classes are successfully immobilized with very high yields on appropriate supports [1 - 3]. These immobilized products are intended for use in construction of biosensors or bioreactors, and also for creation of biomimetic catalysts - synthetic analogues of enzymes or enzymes-like systems are one of the perspective directions in catalase sphere [4 - 6]. Researchers have paid much of their increasing attention to polymeric catalysts containing transitive metals.

Similar catalysts can be considered as the proximal analogues of metalloenzymes, which are expected to show a high catalase activity. It is well recognized that porphyrins are π -conjugated macrocycles, which play a vital role in living organisms [7]. They are constituent parts of many enzymes, vitamins. Porphyrins are known to play a significant role in several biological systems. For example, the presence of such complexes is essential for the activation and storage of oxygen (haemoglobin and myoglobin) as well as for solar energy transfer (chlorophyll) [8]. Metal complexes attached to synthetic polymer matrices often show a specific behavior in the binding reaction of small molecules, because the reactions are affected by the polymers that surround the complex moieties [9].

High-swelling hydrogels are rather perspective in catalysis; they consist of polymeric circuits where the functional groups are bound by covalent bonds forming thus spatial structures. It is possible to get various structure transient metal complexes on the basis of such hydrogels especially in view their ability to swelling which provides high reagents permeability. Furthermore, the polymer net mobility provides to direct the catalyst to a substratum movement. The nature of linear macromolecules interaction and their low-cross-linked analogues with metal ions can be ionic, coordination or ionic and coordination [10]. It is defined by the state of the macromolecules functional groups.

The main aim of this work is to prepare a new bioactive support appropriate for immobilization of bioactive motifs through different interactions in view of their further practical application.

EXPERIMENTAL

Materials

Synthetic polymers polyethylene imine (PEI) and various salts of iron (III) of "c.p." qualification were used without additional purification.

Ferro- and ferricyanide potassium were recrystallized following the procedure described in ref. [11]. The acidic solutions of $K_4[Fe(CN)_6]$ required working under traction and using rubber gloves. 110 g of $K_4[Fe(CN)_6] \cdot 3H_2O$ (technical) were dissolved in 150 ml of water. The solution was evaporated until its density became 1.2. Then 0.1 g of activated carbon was added, stirred and, after settling, was filtered for 10 min. The crystals that precipitated upon cooling the filtrate were sucked off on a Buchner funnel, washed with 5 ml of water and dried at room temperature. The yield was 70 g - 73 g (65 %).

The salt $K_3[Fe(CN)_6]$ was obtained by recrystallization of the technical one. 130 g - 135 g of $K_3[Fe(CN)_6]$ (techn.) were dissolved in 350 ml of water with stirring and heating not exceeding 700°C. The solution was filtered and cooled. The precipitated crystals were aspirated off on a Buchner funnel, washed with a small amount of water and dried at room temperature. The yield was 50 g (37 % - 38 %). With repeated recrystallization using mother liquors to dissolve the salt, the yield could be increased to 80 % - 90 %.

The gel of polyethylene imine (PEI) was synthesized by the method described in ref. [12] - from 10 % solution of the previously prepared polymer in dimethylforma-

mid medium in the presence of epoxy crosslinker — epichlorohydrin at a temperature of 338 K to the point of gelation. The resulting crosslinked polymer was treated with 0.1 M hydrochloric acid and repeatedly washed for two weeks with a large volume of water.

Polyethylene imine hydrogel (PIH) complexes and ferrous salts connected with the catalase structure that has the so-called "natural mark" in the prosthetic group — hemo iron were chosen for the study.

Interpenetrating polymer meshes based on natural agar-agar polymer and synthetic polymers of polyacrylic acid, polyacrylamide, polyethyleneimine synthesized in the laboratory were also used.

Preparation of Polymer Network (IPN)

Interpenetrating polymer networks based on natural agar-agar polymer and synthetic polymers were obtained by polymerization of acrylic acid in agar-agar solution at 70°C [13]. Agar-agar samples of 1.0 g each were dissolved in 50 ml of water and heated to 40°C, then 10 ml of acrylic acid (IPN 1), 10 g of acrylamide (IPN 2) and 10 ml of polyethylenimine (IPN 3) were added to the resulting clear solutions. 0.15 g of MBAA was added as a cross-linking agent. This was followed by the quick introduction of 0.5 ml of $Na_2S_2O_3$ and 0.5 ml of $K_2S_2O_8$. The resulting solutions were adjusted to 100 ml. The polymerization was carried out within 1 h at 70°C.

The IPN matrix was prepared in the shape of cylinders. They were cut into disks, and then immersed in water and left for several days to wash out any residual monomer. After washing IPN was left to dry at room temperature [7].

Extraction of Porphyrin (phaeophytin-b): The first part of the porphyrin was extracted from oil but because of the small amount, it was decided to take it from plant raw materials. For the next step porphyrin (mix of phaeophytins a and b) (Php) was extracted from plant raw materials - a nettle (folia urticae (dioicae)). The method was described in detail in ref. [14]. For the preparation of a different concentration of phaeophytin solution an exact amount of Php was dissolved in an alcohol.

The complexes of transition metal ions with polymeric hydrogels were obtained by mixing pre-swelled hydrogels with solutions of salts of different concentrations. To do this, samples of gels of the same mass (0.01 g) were introduced to 10 ml of distilled water and held for 24 h. Then solutions of salts of transition metals of a definite

concentration were added to the swollen gels samples and left for 2 h - 4 h to reach an equilibrium in the system. The ternary complex GPEI-Men⁺-DBSNa was obtained as follows. Sodium dodecylbenzyl sulfate, 10 ml solutions of copper (II) chloride of different concentrations were added to 0.01 g of the hydrogel. Fe³⁺-phaeophytin (Fe-Php) was immobilized on the IPN via coordination and ionic bonds in an alcohol solution.

Methods

The networks swelling factor was defined through gravimetry using the following formula:

$$R_s = \frac{m_1 - m_0}{m_0},$$

where m_1 and m_0 were the masses of the swelled and dry network (g), correspondingly.

The quantitative determination of interactions between porphyrins and polymers was done using UV-Vis spectroscopy. The liquid over the hydrogel was analyzed at $\lambda = 410.5$ nm.

The binding degree of Fe-Php by IPN was determined as a ratio of the solution concentration determined after the reaction to the initial concentration of Fe-Php. The equation used was as follows:

$$\theta = \frac{(C_0 - C)}{C} \times 100$$

where θ was the binding degree [%], C_0 was the initial concentration of Me-Php (mol/l), while C was the concentration of Me-Php solution determined by analyzing the liquid over IPN after adsorption (mol/l) [14].

The solutions pH values were measured by pH-meter OP-264/1 («Reanal», Hungary) with an accuracy of 0,03 pH units. The complexes catalase activity was measured by the permanganometry method. The procedure applied was as follows. 10 ml 0,1 M of phosphatic buffer were introduced at pH of 7,0 to a thermostatic vessel (25°C). Then the complex batch of a weight of 20 mg was added and mixed. 2 ml 0,12 M of a hydrogen peroxide solution were poured in the course of the mixing. Three minutes later the complex batch was screened through a Shot filter and 5 ml of 20 % sulfuric acid solution were added. The hydrogen peroxide surplus available at the test procedure was titrated with 0,025 KMnO₄ solution. The catalase activity of the complex was measured by

the technique described in ref. [6].

Threefold complex of PIH-Cu²⁺-DBCNa was prepared by the addition to the batch complex of polyethylene imine hydrogel of copper (II) nitrate solution, followed by sodium dodecylbenzylsulphate water solution (DBCNa) aiming the ratios of 4:1:2, 4:1:1 and 4:1:0,5. The complexes obtained were washed with distilled water. Then their catalase activity was determined.

RESULTS AND DISCUSSION

Researchers have recently paid much of their attention to biomimetic problems. In fact, biomimetics studies systems which imitate all the animate nature process, including various functions, such as biocatalytic ones. Special attention is given to the enzyme-like systems creation. The latter work like metal-containing enzymes and have almost the same characteristics in respect to their activity and selectivity. Therefore, up till now the researchers have been interested in model systems simulating catalase enzyme activity and catalyzing hydrogen peroxide decomposition. There are three important reasons for this increased attention: (i) catalase is one of the most effective enzymes with a great number of revolutions; (ii) it is possible to reach an activity close to that of catalase through an appropriate model creation process; (iii) like in case of catalase it is possible to track the iron ion ligand environment influence as well as to define a role of apoenzyme in the catalyst activity [15].

A slightly cross-linked polyethylene imine hydrogel is an object of research. Besides, the researchers are very interested in studying the physical and chemical properties of polyethyleneimine because of its great practical value. The complex-forming ability of polyethylene imine hydrogel is attributed to the presence in its circuit of iminogroups being linked with metal ions in the course of donor-acceptor interactions. The ion of a metal with a vacant d-orbital is the acceptor, while the donor is the nitrogen atom having a pair of electrons to provide the interaction [14]. For complexes-formation of polyethylene imine hydrogel with metal ions it is necessary to provide a deprotonization process of polymeric circuit N⁺H₂-groups. It is possible if ions of transient metals are capable to compete with protons for donor-acceptor interaction with a non-divided pair of nitrogen electrons. It is supposed that the reaction mechanism of complexes-formation corresponds to the

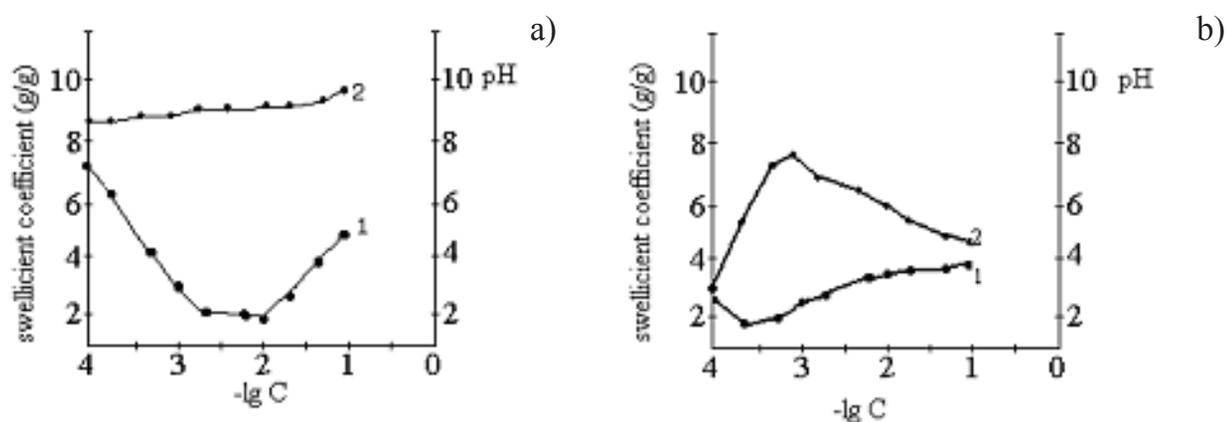
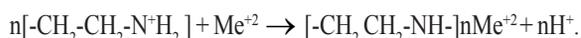


Fig. 1. Dependence of swelling factor (1) of polyethyleneimine hydrogel and solution pH (2) from the salt concentration: a) $K_4[Fe(CN)_6]$, b) $K_3[Fe(CN)_6]$.

replacement process of protons by metal ions according to the following scheme:



Aiming enzyme-like systems creation, we have received polyethylene imine hydrogel complexes with various bivalent and trivalent ferric iron salts. See Fig. 1a, where we show the dependences of polyethylene imine hydrogel swelling factor and subgel solution pH on the concentration ratio of potassium ferrocyanide. The swelling factor (R_s) decreases with increase of the salt concentration and goes through a minimum at a concentration of $7,5 \cdot 10^{-3}$ M. This indicates potassium ferrocyanide complex formation with the participation of the iminogroups of polyethylene imine hydrogel in a ratio of 1:4. In this case the volumetric parameters of the macromolecules [15] are decreasing, while the solution pH varies insignificantly.

For the polyethylene imine hydrogel - $K_3[Fe(CN)_6]$ system (Fig. 1b) there is an antipate change of the swelling factor and solution pH within the concentration range studied. S_d minimum is shifted to the area of lower salt concentration values. The solution pH increase can be attributed to the allocation of hydroxyl ions as a result of the ionic exchange reaction between the complex ferrous anions and the hydroxyl groups of the protonated polymeric ligand nitrogen atoms. The superstoichiometric ratio of the functional groups of the hydrogel to the ions $[Fe(CN)_6]^{-3}$ and $[Fe(CN)_6]^{-2}$ leads to an increase of the osmotic pressure in the mesh due to the free ferrous ions and their counterions that

do not directly participate in the sequestering process. In view of the polyethylene imine hydrogel polymeric ligand nature it is assumed that the macromolecules of the nitrogen-containing polymer compete with Fe^{+2} and Fe^{+3} in the course of the coordination circuit formation because of cyanide ions displacement out of the ferrous ion coordination sphere. It is proved by the fact that the hydrogel changes from colorless to green.

The biocatalase properties of polyethylene imine hydrogel - $K_4[Fe(CN)_6]$ and $K_3[Fe(CN)_6]$ complexes observed in the reactions of hydrogen peroxide decomposition process (Fig. 2) present a greater interest. The activity of polyethylene imine hydrogel complex with potassium ferricyanide is higher than that of the complex with potassium ferrocyanide. The maximal activity is

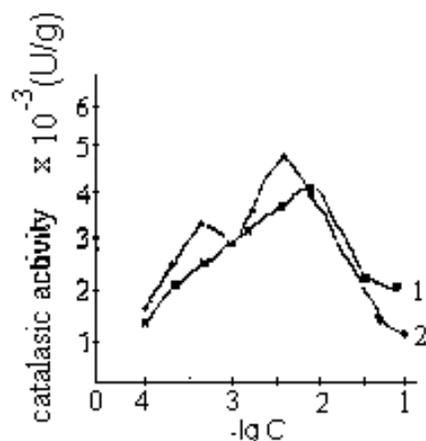


Fig. 2. Dependence of PIH catalase activity complexes - $K_4[Fe(CN)_6]$ (1) and PIH - $K_3[Fe(CN)_6]$ (2) according to the component ratio.

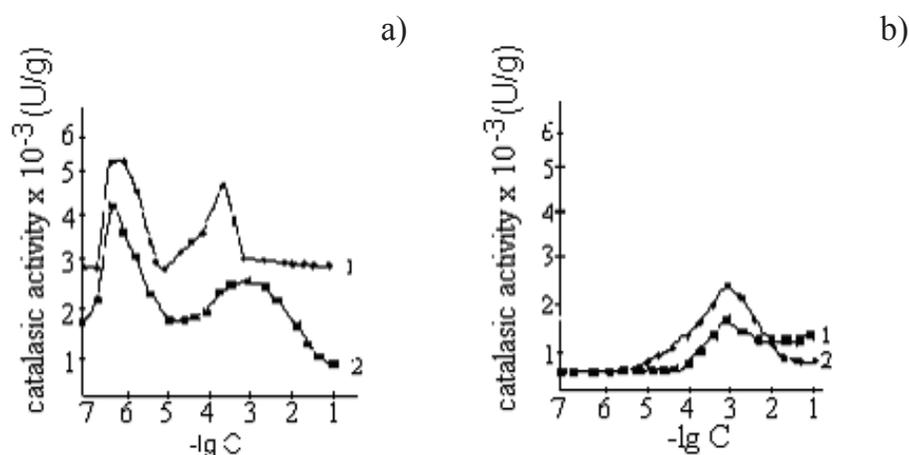


Fig. 3. Dependence of catalase activity of PIH complexes with ferrous salts from salt concentration: a) PIH – Fe(NO₃)₃ (1) and PIH – FeCl₃ (2); b) PIH – Fe(CH₃COO)₂ (1) and PIH – FeSO₄ (2).

observed at the salt concentration of $1,0 \cdot 10^{-2} \text{M}$.

The interaction of potassium ferricyanide with the polymer results in the formation of polymer-ferricyanide complexes. It is assumed that the catalase activity of the systems is predominantly attributed to Fe (III) and Fe (II) complexes. The latter ions have the role of a reservoir filling Fe (III) losses within the framework of catalase process [16].

The interaction process of polyethylene imine hydrogel with ferrous neutral salts of a varying concentration is studied aiming a comparison with the catalase activity of PIH complexes with Fe⁺³ and Fe⁺² salts (Fig. 3). It is evident that the catalase activity depends on the metal ion valency, while the salt anion has no effect. This observation is in accord with the findings reported in ref. [5].

The threefold PIH-metal-SAS complexes are of

a great interest in view of elaboration of enzyme-like systems. The activity of polyethylene imine hydrogel complexes containing copper content (II) and sodium dodecylbenzylsulphate (DBCNa) is studied. The greatest catalase activity is shown by the complexes containing the components ratio of 4:1:1. At a constant substratum concentration the increase of the catalyst content leads to increase of hydrogen peroxide decomposition process. It is connected with the increase of the share of the active centers in the reactionary mixture. At a constant catalyst weight the substratum concentration increase leads to decrease of the complex catalase complex (Fig. 4a); the catalase activity does not practically change in case of the highest substratum concentration. Besides, the solution pH affects the rate of hydrogen peroxide decomposition very slightly at the beginning of the process (Fig. 4b).

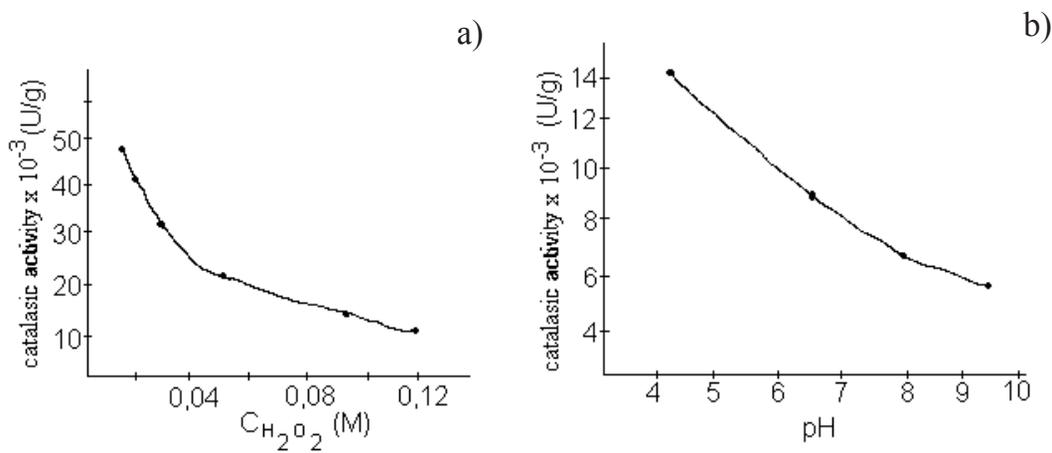


Fig. 4. Dependence of CA of threefold complex PIH-Cu²⁺ - DBCNa 4:1:1 upon hydrogen peroxide concentration (a) and solution pH (b): a) t = 3 min, pH = 7.0, T = 25°C; b) t = 3 min, C(H₂O₂) = 0.12 M, T = 25°C.

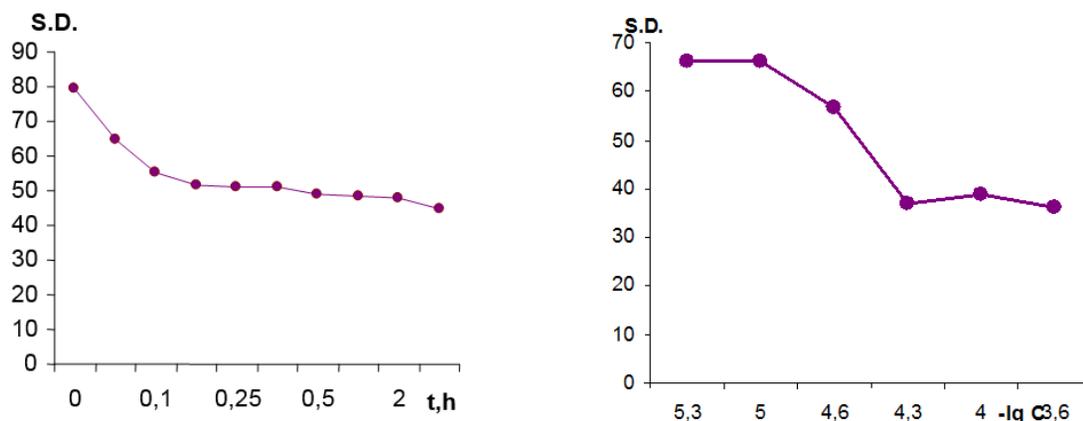


Fig. 5. Swelling degree (%) as a function of time for IPN at constant concentration of Fe-Php (a) and as a function of $-\log C$ (Fe-Php).

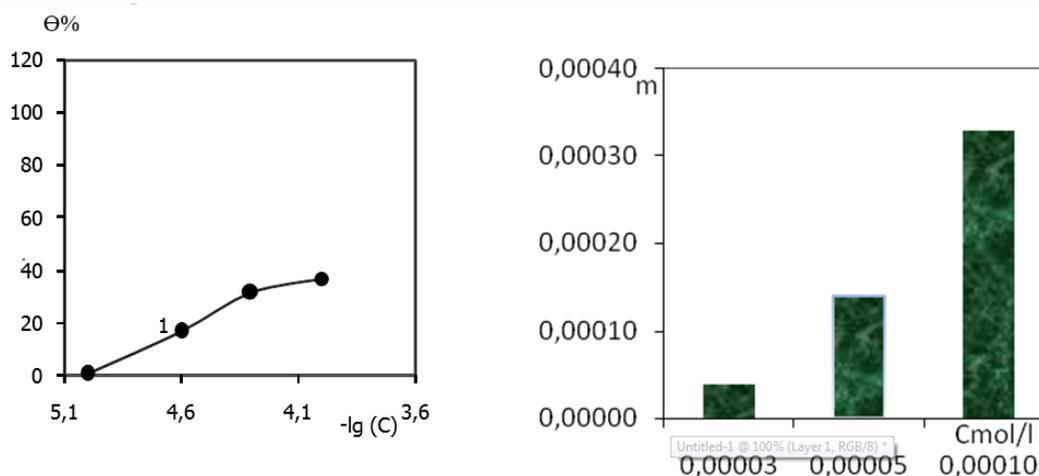


Fig. 6. The amount m (g) of adsorbed Fe-Php by IPN as a function of $-\log C(Fe-Php)$.

The role of sodium dodecylbenzylsulphate in accelerating the hydrogen peroxide decomposition process is the following. The PIH-Cu complex electrostatic interaction with the detergent leads to macromolecules compactification as well as to an increase of the local concentration of the active centers in the macromolecular cluster [17]. Probably, the group formation with the participation of enzymes and hydrophobic units is one of the most important conditions for effective catalysts formation process.

The same investigations are carried out with IPNs based on Ag-Ag - PI. The synthesis of the IPN matrix is carried out aiming to determine its swelling kinetics and complex formation properties in relation to Fe-phaeophytin. The results of the complex formation study show a coun-

teraction of the swelling degree, which in turn suggests specific interactions between IPN and Fe (III)-Php (Fig. 5).

The quantitative determination of the interactions between porphyrins and polymers are carried out spectroscopically. The liquid over the hydrogel was analyzed at $\lambda = 410.5$ nm. The binding degree of Fe-Php by the IPN is calculated using the equation pointed above. The immobilization results are presented in Fig. 6. An increase of the porphyrin concentration in the adsorption medium leads to an increase in the binding degree. Immobilization on the IPN matrix based on Ag-Ag and PI with a binding degree of 40 % (Fig. 6a) and adsorbed Fe (III)-phaeophytin of 0.0003 g (Fig. 6b) is achieved. This phenomenon may be explained by the rigidity of the natural Ag-Ag chain of IPN.

CONCLUSIONS

The interaction of transition metal ions and their complex anions is investigated. Polymer complexes of a different composition are obtained. Besides, a ternary complex of PIH containing a copper ion and a surface-active substance (sodium dodecyl benzyl sulfat) is received. The biocatalytic activity of these complexes in the course of decomposition of hydrogen peroxide, a substrate of enzyme catalase, is studied for the first time. It is shown that the activity of the iron containing complexes depends on the valence state of the metal ion – the activity of the complexes containing ferric ions is higher. The presence of a surface-active substance in the ternary complexes PIH – Cu(II) – DBSNa leads to the formation of more compact macromolecules and therefore to increase of the local concentration of the active centers in macromolecule coil followed by acceleration of H₂O₂ decomposition. The quantitative determination of the interactions between porphyrins and IPN is carried out spectroscopically by analyzing the liquid over the hydrogel. The experimental data obtained leads to the conclusion that the complex formation with the participation of porphyrin and IPN is hampered. It can proceed along different mechanisms. The PIH interactions with transition metal ions including their complex anions are also investigated. Researches of the complexes biocatalytic activity have shown that their activity is influenced by the metal ions valency, the hydrogel swelling and the initial components ratio. The swelling and adsorption ability of the matrix as well as the binding degree of IPN with Fe-Php are also reported. The swelling kinetics of IPN matrix is studied.

It is concluded that the swelling of the hydrogels determines the reagents high permeability. The mobility of the polymer network ensures the “adjustment” of the catalyst to the substrate. The use of the modeling method for the study of catalysts, whose action is based on the principles of enzymatic systems, is productive, primarily because such artificial systems, being of a relatively simple structure, are available for detailed physical and chemical studies. Thus, as a result of the study, new knowledge on the mechanism of action and the structure of the active center of enzymes can be obtained. In addition, the control of the polymer gel swelling provides to control the catalytic process. The attractiveness of such systems for biotechnology and medicine is obvious. Analogical systems are already used for the extraction of metals from natural and waste water.

REFERENCES

1. Y. Leanirith, C. Bunel, J.P. Vairon, Reversible immobilization of drugs on a hydrogel matrix, 2. Diffusion of free chloramphenicol from poly(2-hydroxyethyl methacrylate) hydrogels. *Die Makromolekulare Chemie*, 191, 1990, 1119-1129.
2. J.W. Lee, S.Y. Kim, S.S. Kim, Y.M. Lee, K.H. Lee, S.J. Kim, Synthesis and characteristics of interpenetrating polymer network hydrogel composed of chitosan and poly(acrylic acid), *Journal of Applied Polymer Science*, 73, 1999, 113-120.
3. J. Berger, M. Reist, J.M. Mayer, O. Felt, N.A. Peppas, R. Gurny, Structure and interactions in covalently and ionically crosslinked chitosan hydrogels for biomedical applications, *European Journal of Pharmaceutics and Biopharmaceutics*, 57, 2004, 19-34.
4. D.I. Metelitsa, Modelling of redox enzymes, *Minsk, Science and technology*, 1984, 892, (in Russian).
5. G.A. Bektenova, *Izvestiya, MES RK*, 3, 2001, 82-91, (in Russian).
6. A.E. Shilov, Biomimetic catalase, New materials and technologies, *Innovations of XXIc, Research activities at Moscow Institutes, Conference proceedings, Chernogolovka, 1-4 oct., 2001, Chernogolovka: Publishing house IPHF, RAS-2001, Chemical series, 27-28, (in Russian).*
7. T.K. Botabekova, J.S. Kramorenko, I.S. Stepanova, A.A. Esimova, N.M. Zhunusbekova, Experimental investigation of phaeophytin activity as photosensitizer, *Materials of the scientific congress of ophthalmologists «Pressing questions of clinic, diagnostics and treatment of eye illnesses», 2008, 281-284, (in Russian).*
8. Y. Xia, T. Guo, M. Song, B. Zhang, Hemoglobin recognition by imprinting in semi-interpenetrating polymer network hydrogel based on polyacrylamide and chitosan. *Biomacromolecules*, 6, 2005, 2601-2606.
9. G. Pozniak, B. Krajewska, W. Trochimczuk, Urease immobilized on modified polysulphone membrane: preparation and properties, *Biomaterials*, 16, 1995, 129-134.
10. J. Leaka, J. Włodarczyk, W. Zaborska, Polyaniline as a support for urease immobilization, *J Mol. Catal. B Enzyme*, 6, 1999, 549-556.
11. Yu.V. Karyakin, I.I. Angolov, *Pure chemicals, Moscow, Chemistry*, 1974, p. 408.
12. E.E. Yergozhin, E.Zh. Menligaziev, Polyfunctional

- ion exchangers, Alma-Ata, Science, 1986, p. 304.
13. R. Hobzová, N.M. Zhunusbekova, J. Širc, J. Michálek, Surface modification and characterization of methacrylate polymer networks for tissue engineering. 9th Annual Conference of the Yugoslav Materials Research Society 'YUCOMAT', Herceg Novi, 2007.
 14. R. Hobzova, M. Pradny, N.M. Zhunusbekova, J. Širc, V. Guryca, J. Michalek, Bioactive support for cell cultivation and potential grafting. Part 1: Surface modification of 2-hydroxyethyl methacrylate hydrogels for avidin immobilization, *E-Polym. Art.N.* 043, 2011, 474-490.
 15. A.R. Brodskij, I.A. Shlygina, I.S. Chanysheva, V.P. Grigorjeva, V.I. Jaskevich, A.K. Zharmagambetova, S.G. Muhamedjanova, N.Z. Tumabaev, B.S. Selenova, *Tidings of the NAS RK, Chemical series*, 2005, 1, 24-34, (in Russian).
 16. N.M. Zhunusbekova, N.B. Sarova, T.K. Jumadilov, E.A. Bekturov, The complexformation of metalloporphyrin with weakly crosslinked polyacrylic acid, *Chemical Journal of Kazakhstan MES RK*, 1, 2006, 142-147.
 17. R. Hobzová, N.M. Zhunusbekova, J. Širc, B. Dvořánková, K. Smetana, M. Přádný, J. Michálek, Methacrylate based hydrogels for tissue engineering, *International Conference on Advances in Polymer Science and Technology*, New Delhi, 28 - 31.1.2008.