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Tetrapyrrole Macrocycles Complexes as a Basis for the Development of Materials with a Combined Biological Action

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Abstract—Extracoordination reactions of pyridine and piperidine nitrogen bases, and nitrite ion with cobalt complexes of water-soluble porphyrins and tetrasulfophthalocyanine were studied. A method for the modification of polymer systems by immobilization of coordination compounds of tetrapyrrole macrocycles on polymer substrates was developed. Bactericidal properties of the obtained composite films were revealed.

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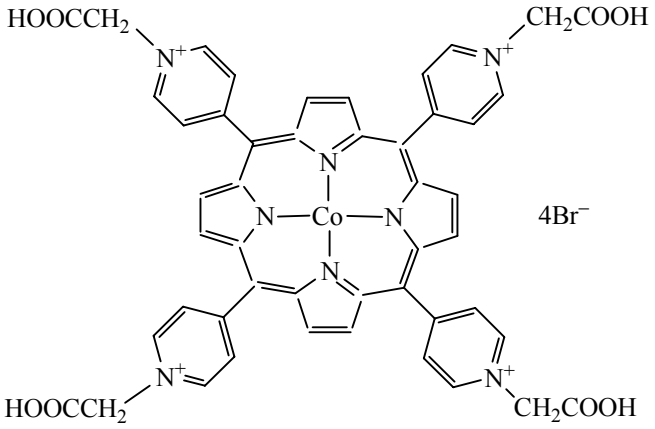
An important task of the modern chemistry is creating new polymeric materials with macroporous structure used as adsorbents for the separation of toxins at the cleaning biological fluids (e.g., in blood hemodialysis). At present, cellulose ethers and certain synthetic polymers (polymethylmethacrylate, polyacrylonitrile, polycarbonate, etc.) are used for this purpose whose sorption capacity and selectivity toward nitrogen-containing biomolecules and ions, which are the blood toxins (urea, uric acid, creatinine, nitrite ion, etc.), is not sufficiently high. One solution to this problem is the immobilization of a biologically active compound on the polymer matrix. Tetrapyrrole macrocycles (porphyrins and phthalocyanines) may be promising objects for solving this problem, because they are the closest structural analogs of bioporphyrins (chlorophyll and blood heme).

Complexes of these compounds with transition metals are capable of attaching additional ligands (extracoordination), and in certain environments, for example, being included in the polymer matrix, they can identify biologically active molecules [1]. Besides, they may act like natural enzymatic systems as selective catalysts in many redox processes [2].

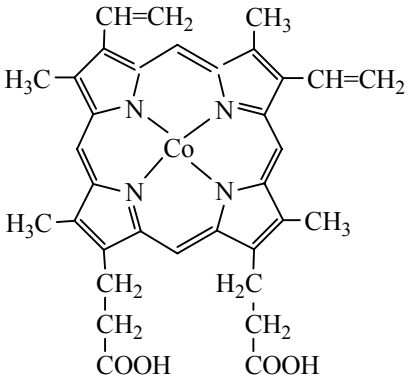
In the present work, to examine potential substances for the modification of polymer systems by coordination compounds of tetrapyrrole macrocycles and simulation of the reactions occurring in biological systems, we performed a study of equilibrium extracoordination reactions of nitrogen bases pyridine (Py) and piperidine (Pip), and nitrite ion with the cobalt complexes of macrocycles of different structures: 5,10,15,20-tetra(4-*N*-carboxymethylenepyridyl)porphyrin tetrabromide (**I**), protoporphyrin-IX (**II**), and tetrasulfophthalocyanine (**III**). The studies were carried out using aqueous and aqueous phosphate buffer solution at pH 7, 6 and 9.0 at 298.15 K.

These porphyrin and phthalocyanine complexes are ionized in water and slightly alkaline solutions (phosphate buffer, pH 7, 6 and 9.0) and can exist as an equilibrium mixture of monomer and associated forms. It is believed, however, that the association of the ionized forms of tetrapyrrole macrocycles in aqueous solutions occurs only at very high concentrations. The dilution shifts the equilibrium to the side of the monomeric form of the complex [3]. In order to prevent the dimerization process, we used solutions with extremely low concentrations of reactants ($c_{CoP} = 10^{-5}$–10^{-6}, $c_L < 10^{-4}$ M) at a pH close to physiological pH. At these concentrations further dilution of the studied compounds did not affect the position of absorption bands in their electronic spectra (Fig. 1), which suggests the presence of the monomeric form.

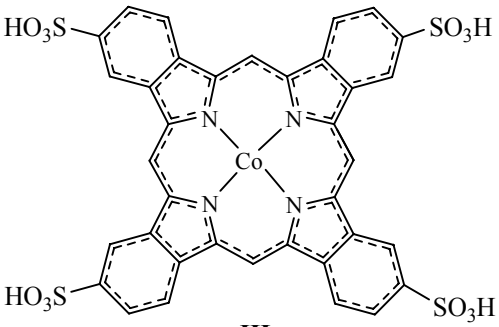
Using the method of spectrophotometric titration, we determined the thermodynamic stability constant (K_{stab}) of the axial complexes of pyridine (Py),



I



II



III

piperidine (Pip), and nitrite ion with cobalt complexes **I–III**. Figure 2 shows a typical example of spectral changes at the titration of complex **III** with a solution of piperidine**.**

In general, the coordination of extraligand by the tetrapyrrole macrocycle cobalt complexes occurs according to Eq. (1):

$$\mathrm{CoP} + n\mathrm{L} \rightleftarrows \mathrm{L}_n\mathrm{CoP},\ n = 1, 2, \qquad (1)$$

where CoP is a complex of the macrocycle with Co^{II}, L_nCoP is axial complex, L is the axial ligand. In aqueous buffer solutions the spectral changes of cobalt complexes at adding Py, or Pip, or NO_2^- include clear isobestic points (Fig. 2). This suggests that the reaction proceeds with the formation of the CoP(L) complexes.

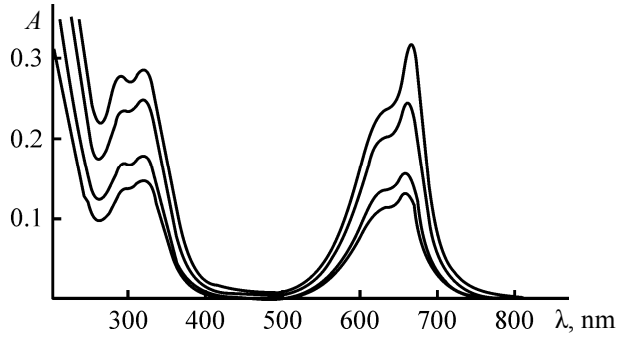


Fig. 1. Changes in the electronic spectra of CoP(III) solutions (pH 7) at the concentrations from 0.33×10^{-5} (bottom) to 0.94×10^{-5} M.

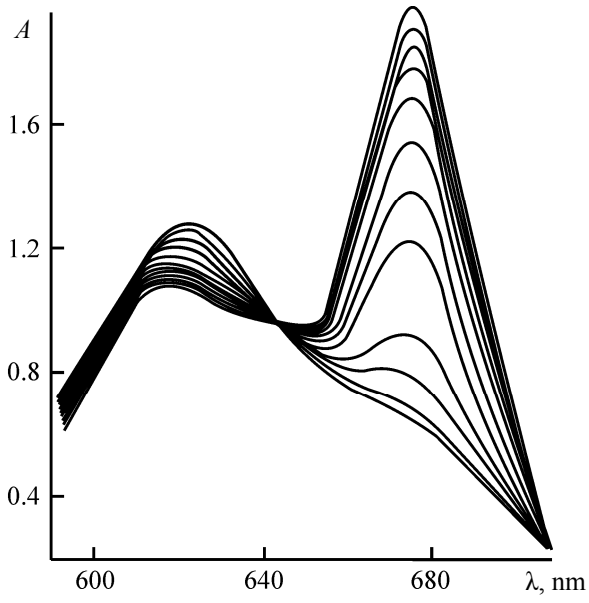


Fig. 2. Changes in the electronic spectra of CoP(**III**) in water at adding extraligand piperidine. Phosphate buffer, pH 7.6, 298 K, $c_{\mathrm{CoP(III)}}$ from 2×10^{-6} M.

Table 1. Stability constants of the axial complexes of compounds **I–III** at 298 K at the analytical wavelength

Porphyrin	λ_a, nm	K_{stab} in water, l mol^{-1}		
		Py	Pip	NO_2^-
I (pH 7.4)	437			26±4
II (pH 9.0)	420			3.9±0.5
III (pH 7.6)	328	14±2	45±10	

Table 2. Parameters of electron absorption spectra of aqueous solutions of compounds **I–III**

Comp. no.	pH	λ_{max}, nm (log ε)				
I	7.6	–		595 sh	540 (4.06)	427 (5.02)
II	9.0			564 (3.98)	534 (4.01)	417 (5.41)
III	7.6	666	633		328	301

Experiments were carried out under conditions where $c_L^0 >> c_{CoR}^0$, and the number of involved ligands n was determined from the slope of the plots log $[(A - A_0)/(A_k - A)]$ vs. log c_L. Within the measuring error, n was equal to unity (Fig. 3). The stability constants of the axial complexes (K_{stab}) calculated from Eq. (2) remained unchanged at the change in the analytical concentration L by an order of magnitude, within the measuring error. The optical density at the analytical wavelengths was used to calculate the equilibrium concentrations of the extracomplex CoP(L):

$$K_{stab} = [CoP(L)]/([CoP][L]), \quad (2)$$

$$c_{LCoP} = c_{CoP}^0(A - A_0)/(A_{fin} - A_0). \quad (3)$$

Here c_{LCoP} is the equilibrium concentration of the LCoP complex, c_{CoP}^0 is the initial concentration of CoP, A_0, A, and A_{fin} are the initial, equilibrium and final optical densities if the solution, respectively.

Piperidine (Pip) is significantly stronger donor of a pair of σ-electrons than Py, which results in a threefold increase in K_{stab} in going from cobalt phthalocyanine tetrasulfoacid pyridine extracomplrx to piperidine extracomplex (Table 1).

The investigation of stability of nitrite complexes of Co-porphyrins showed that **I** forms more stable axial complexes compared with **II** (Table 1). This is probably due to the presence of positive charge on the pyridyl rings of water-soluble porphyrin, which reduces the electron density in the macrocycle and, hence, stabilizes the axial complex. Adding solutions containing nitrite ions to **III** was noted to lead to no significant spectral changes.

Phthalocyanine complexes are known [4] to have a reduced electron density on the central nitrogen atoms compared with porphyrinate complexes and, consequently, the effective positive charge on the central metal cation complexes with phthalocyanine is higher than that in the water-soluble metalloporphyrins. Therefore extracomplexes of metal phthalocyaninates are more stable than the corresponding derivatives of metalloporphyrins.

The observed ability of complex **III** to the additional coordination of the nitrogen-containing ligands gave reason to use cobalt tetrasulfophthalocyaninate for the modification of hydroxyethylcellulose polymer.

In going from aqueous solutions of **III** to the polymer–**III** system no significant changes were observed in the Co-phthalocyanine EAS (the hydroxyethylcellulose did not possess any intense absorption bands in this spectral range). IR spectra of the films confirm the absence of donor–acceptor interactions between the central cation of the tetrasulfophthalocyanine complex and the polymer matrix. Thus, in the composite material the cobalt phthalocyanane complex **III** retains the ability to attach additional ligands by its free coordination site.

The EAS of investigated compounds are given in Table 2.

The study of antimicrobial properties of the initial and phthalocyanine-containing films toward the test cultures of *Escherichia coli* and *Staphylococcus aureus*

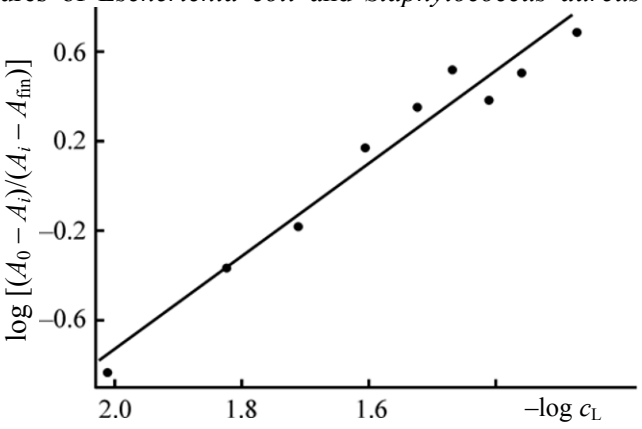


Fig. 3. The plot log $[(A_i - A_0)/(A_{fin} - A_i)]$ vs. log c_L for the systems of CoP(III)–Pip in aqueous solution, pH = 7.6, at T = 298 K.

showed that the modified films have a depressing effect on bacterial cells of both types, that is, they can be used as an antiseptic material.

EXPERIMENTAL

Electron absorption spectra were measured on a Specord M-400 and a Hitachi U-2000 spectrophotometers in polished quartz cells at 298 K. IR spectra of polymer films were recorded on an Avatar 360 FT-IR ESP spectrophotometer in the range 400–4000 cm^{-1}.

The investigation of the processes of extracoordination of piperidine, pyridine, and NO_2^- by the cobalt porphyrin and phthalocyanine complexes was carried out spectrophotometrically on the Specord M-400 instrument in 50 ml cells (thickness of the absorbing layer 100 mm) at 298.15±0.10 K. A specified volume of an extraligand solution was added to a solution of metalloporphyrin using microsyringe, the dozing error did not exceed 0.01%. The increase in volume of the solution in the cell after completion of titration did not exceed 0.05%.

The solvents (pyridine and piperidine) were preliminarily purified by the methods described in the literature [5, 6]. $NaNO_2$ of chemically pure grade was purified by double recrystallization [7]. Phosphate buffer solutions were prepared according to [6].

5,10,15,20-Tetra(4-*N*-carboxymethylenepyridyl)-porphyrin tetrabromide (I) was prepared according to the procedure published previously [8]. The Co-protoporphyrin **IX** complex was synthesized by boiling protoporphyrin **IX** dimethyl ester with an excess of cobalt acetate in acetic acid followed by alkaline hydrolysis and precipitation with dilute hydrochloric acid [9], yield 90%.

Cobalt phthalocyanine complex III ($CoC_{36}H_{20}N_4O_{12}S_4$) was prepared by the condensation of sulfophthalic acid with urea and cobalt chloride in the presence of a catalyst by the method described in [10, 11] The complex was purified by reprecipitation from concentrated sulfuric acid followed by extraction of impurities soluble in organic solvents in a Soxhlet apparatus using acetone and ethanol. EAS (DMSO), λ_{max}, nm (log ε): 665 (5.2), 603 (4.5), 330 (4.9).

The polymer carrier was Klucel hydroxyethylcellulose, USA. Aqueous polymer solutions with concentrations from 0.2 to 2 wt % were prepared gravimetrically. The modification of the film was performed by introducing the polymer into the aqueous solution of cobalt tetrasulfophthalocyaninate **III** complex. The polymer film was obtained by casting a 2% aqueous solution of polymer onto a glass substrate. The film samples were dried to a constant weight. The film thickness was 70 μm. The modified hydroxyethylcellulose film was transparent and had a light blue color.

Test on the bactericidal properties of the films in relation to *Staphylococcus* and *E. coli* was performed in Petri dishes by a *seed* method, according to [12].

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