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Magnetic and Spectroscopic Methods for Structural Characterization of Paramagnetic Hematoporphyrin IX Complex With Cu(II)

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Abstract

A paramagnetic metaloporphyrin type complex [Cu(II)(Hp_{-2H})].2H_2O has been obtained during the reaction of Cu(II) and hematoporphyrin IX ((7,12-bis(1-hydroxyethyl)-3,8,13,17-tetramethyl-21H-23H-porphyn-2,18-dipropionic acid) - Hp, **I**) in alkaline aqueous medium. The solution and solid state structure of the complex were studied through UV-Vis, IR and EPR spectroscopic methods. The applied simulation procedure using the parameters from the experimental EPR spectrum proved the square planar structure of the complex with symmetrical coordination of the porphyrin ligand to Cu(II) via the four pyrrole N-atoms in the porphyrin macrocycle.

Keywords: hematoporhyrin IX, metalloporphyrin of Cu(II), EPR studies, IR, UV-Vis spectra

1. Introduction

Recently a particular interest has been shown towards the complexes of hematoporphyrin IX with transition metals, due to their application in different areas [1- 4] here including their use as photo- and radiosensitizers. A powerful impact in this research is the proved tendency of the porphyrin molecules to become localized in rapidly growing neoplastic tissues [5 - 7]. Thus an improvement of the cellular uptake of the platinum anti-cancer drugs could be achieved by their conjugation with porphyrin molecules which provide an active transport and tissue specificity [7 – 11]. In this respect a large number of papers have appeared dealing with synthesis, structural characterization and cytotoxic activity investigation of Pt(II) complexes with hematoporphyrin and hematoporphyrin type ligands [7, 10 - 15]. In most of these compounds [7, 10 – 13], platinum(II) species is attached to the propionic acid side chains out of the porphyrin moiety. For such porphyrin complexes a cytostatic activity is expected derived from platinum component and additional toxicity – upon irradiation – from the porphyrin fragment.

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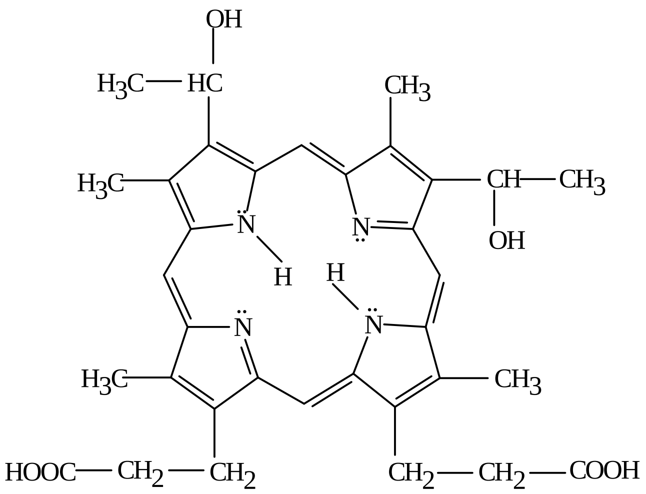
A remarkable feature of hematoporphyrin IX is to stabilize metal ions in intermediate oxidation states such as Pt(III), Pd(III) and Au(II) [13 – 16]. Three stable Pt(III), two Pd(II) and one Au(II) –hematoporphyrin IX complexes with distorted octahedral structure were synthesized in order to estimate their cytotoxicity. The metal ions in all complexes have an octahedral coordination with N, O- hematoporphyrin donor atoms and additional ligands such as NH_3, H_2O, Cl^-. The complexes showed a promising cytotoxicity comparable and in any cases higher than that of *cisplatin* in *in vitro* test against a panel of human leukaemic and lymphoma-derived cell lines. The design and synthesis of these complexes represent a new approach for a development of the cytotoxic agents with improved properties [13, 15].

The acid-base properties of hematoporphyrin IX - porphyrin with peripheral carboxylic acid groups that can dissociate and its metalloporphyrins are the ground for the studying the processes of their interaction with lipid membranes. Kepczynsky et al. [4] have investigated the pH influence on the interaction of Zn-hematoporphyrin IX with liposomes. The cobalt hematoporphyrin IX complex was immobilized on a composite membrane or incorporated with silica gel into a carbon paste electrode in order to study its catalytic activity [2, 3].

Although the hematoporphyrin complexes of the transition metals are well-known long ago [17 – 19] most of the investigations are directed to kinetic and thermodynamic characterization of the equilibrium coordination reactions in solution [17, 20] as well as to study some properties of the complexes thus obtained. Moreover in our knowledge till now there were no examples for solving of hematoporphyrin complexes structures using X-ray diffraction analysis, the most probably because of the difficulties to obtain single crystals as a consequence from the pH-dependant aggregation phenomena [4, 21]. The aim of the present paper was to characterize the solution and solid-state structure of the copper-hematoporphyrin IX complex using magnetic and spectroscopic methods. This complex was obtained in alkaline aqueous medium where the coordination reactions are very often accompanied with a parallel redox processes leading to the formation of hematoporphyrin complexes of metals in uncommon oxidation states [13 – 16]. Despite of the fact that the Cu(II)-Hp interaction at the same conditions is complicated with parallel redox process too, in the solid state hematiporphyrin complex thus obtained copper is in the oxidation state +2.



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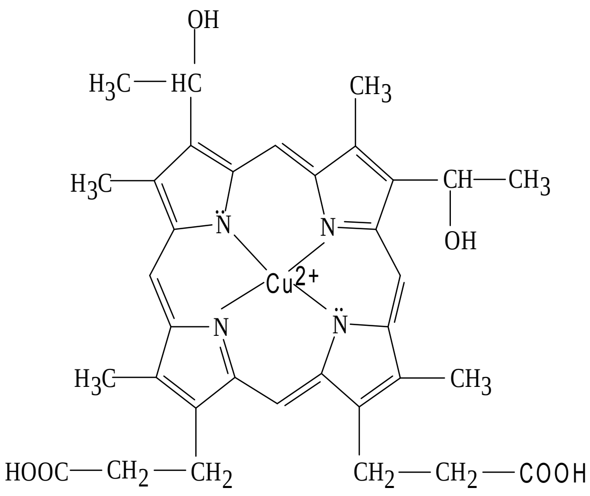

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2. Experimental

2.1. Syntheses

All chemicals were of analytical grade and were purchased from commercial sources, and used without further purification.

A sample of 0.0600 g (0.10 mmol) hematoporphyrin (Fluka) was dissolved by stirring in 8 cm^3 1.10^{-1} M KOH (Merck) (0.8mmol, molar ratio OH^-:Hp=8) until a pH value of 11.0 ÷ 11.5 is obtained. An aqueous solution of $Cu(NO_3).3H_2O$ (Merck, 0.0242 g, 0.1 mmol) was then added in equimolar ratio (Hp:Cu=1) and the basicity of the reaction mixture was adjusted to pH=11.5 – 12.0 using 1.10^{-1} M KOH solution. The reaction system was then kept at ambient temperature for 5 days. During the first day the acidity of the reaction solution increases spontaneously to pH~9.0 - 9.5. A dark violet complexes with composition $[Cu(II)(Hp_{-2H})].2H_2O$ was then isolated from the reaction system by adding of 4 cm^3 0.05 M HNO_3 (Merck). The precipitate was filtered, washed with water and alcohol and dried over P_4O_{10}. Anal. Found: C, 58.40; H, 5.88; N, 7.98; Cu, 9.32 %. Calculated for $C_{34}H_{40}N_4O_8Cu$ (MM 696.3 g/mol): C, 58.65; H, 5.80; N, 8.04; Cu, 9.13%.

The magnetically diluted Cu(II)-Hp complex in a diamagnetic matrix of Zn(II)-Hp was obtained using the procedure similar to that already described above for the magnetically concentrated complex $[Cu(II)(Hp_{-2H})].2H_2O$. For the synthesis an aqueous solution of $Zn(NO_3)_2.6H_2O$ (Fluka, 0.0268g, $9.0x10^{-2}$ mmol) and an aqueous solution of $Cu(NO_3).3H_2O$ (0.0024g,$1.0x10^{-2}$ mmol) together with an alkaline aqueous solution of hematoporphyrin (0.0600 g, 0.10 mmol) were mixed at ambient temperature and using 1.10^{-1} M KOH the starting pH value was again adjusted until 11.5-12.0. Five days later the obtained magnetically diluted hematoporphyrin complex suitable for EPR analysis, with metal composition Zn:Cu=9 was isolated by adding of 4 cm^3 0.05 M HNO_3 (Merck). The precipitate was filtered, washed with water and alcohol and dried over P_4O_{10}.

The Cu(II)-Hp interaction in alkaline aqueous medium was followed potentiometrically, spectrophotometrically and by the EPR method. The electronic spectra were recorded after dilution of the sample from the reaction system with distilled water to 6.25×10^{-5}M in respect to the ligand concentration while the EPR spectra of the reaction system were recorded without dilution.

2.2. Analyses and physical measurements

C, H and N analyses were performed on VARIO ELIII. The copper content was determined using flame atomic absorption spectrometry (FAAS) with an air-acetylene flame (PYE UNICAM SP 1950). The samples for the FAAS analyses were prepared through decomposition with conc. H_2SO_4 and 30 % H_2O_2.

The potentiometric measurement were performed using a pH-meter Radelkis OP-208.The absorption electronic and IR spectra (KBr-disks, 4000-400 cm^{-1} and CsI-disks, 400-200 cm^{-1}) were recorded on UV-Vis - Carl-Zeiss, Jena, Lambda 17 UV-VIS and FTIR-Bruker IFS 113 V and - Perkin Elmer 983 - spectrometers respectively. The EPR spectra were obtained on a X-band - Bruker B-ER 420 - spectrometer in the temperature range 100 - 298K.

3. Results and Discussion

The Cu(II)-hematoporphyrin interaction in alkaline aqueous medium started with a slow gradually decrease of the pH value (Figure 1). The ligand for the syntheses was dissolved in 1×10^{-1}M KOH. The pH value of the reaction system, obtained after the mixing of the ligand and $Cu(NO)_2$ solutions in equimolar ratio was adjusted in the range 11.5-12.0 by addition of 1×10^{-2}M KOH. During the period of the interaction the acidity spontaneously increased with ~2.5 pH units.

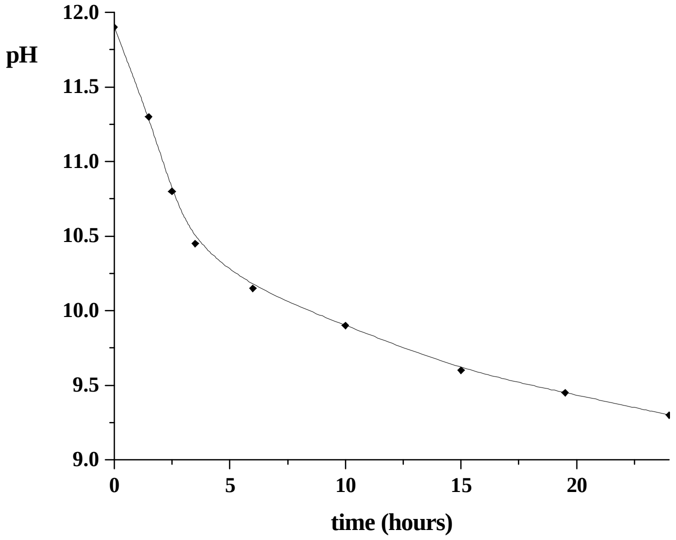

Fig. 1. Potentiometric data obtained for the interaction Cu(II)-hematoporphyrin in alkaline-aqueous medium.

Along with the acidity changes, the observed changes in the electronic absorption spectra proved the metal coordination to pyrrole N atoms in the porphyrin framework (Figure 2). The free ligand dissolved in alkaline-aqueous medium exhibits (Figure 2a) the characteristic Soret band at 374 nm and an etio type distribution in the intensities of the satellite Q-bands being IV>III>II>I (516, 524, 577, 628 nm). Two different processes could be observed following the interaction. The reaction started with a slight hypsochromic shift and decrease in the intensity of the Soret band (369 nm) in comparison with the free ligand spectrum (Figure 2a-c). During this stage a significant alteration in the Q-bands intensity was observed in the spectrum received a day after the reaction start (Figure 2c). A sequence of IV<III<II>I for the satellite Q-bands intensity is observed. In addition, a new band with growing intensity on the whole course of reaction appeared at 243 nm. These spectral changes suggest that the porphyrin ring in the first reaction product is distorted, most probably because of an asymmetrical coordination to copper through two adjacent pyrrole N atoms [13] and formation of a sitting atop type complex. Further spectral changes in the reaction course followed the drop of the pH value. The second stage is characterizes with a red shift of the Soret band to 384 nm and a collapse of the four-banded spectrum to yield two absorption Q-bands (533, 569 nm). At the end of the reaction, all absorption bands have shown a hyperchromic change (Fig. 2e). The final spectral changes observed could be due to a higher symmetry of the porphyrin ring and proved the coordination of copper to the four pyrrole N atoms in the porphyrin macrocycle. Hence the final product from the Cu(II)-Hp interaction is a metalloporphyrin type complex.

The EPR spectra recorded during the interaction proved the diamagnetic character of the copper complexes obtained in solution. An anisotropic weak signal with $g_{\perp}=2.155$ and $g_{\parallel}=2.325$ was only observed, which belongs to the initial solvate Cu(II) species. This signal with decrease intensity was found during the reaction course and most probably is due to the equilibrium concentration of Cu(II) species. During the coordination reaction a signal which could be assigned to the paramagnetic Cu(II)-hematopoprhyrin complex was not observed. On the basis of these results together with the considerable decrease of pH it can be concluded that the Cu(II) and Hp interaction is complicated by a parallel redox process. The final product obtained is a diamagnetic metalloporphyrin-type complex, in which copper most probably is in an oxidation state +1.

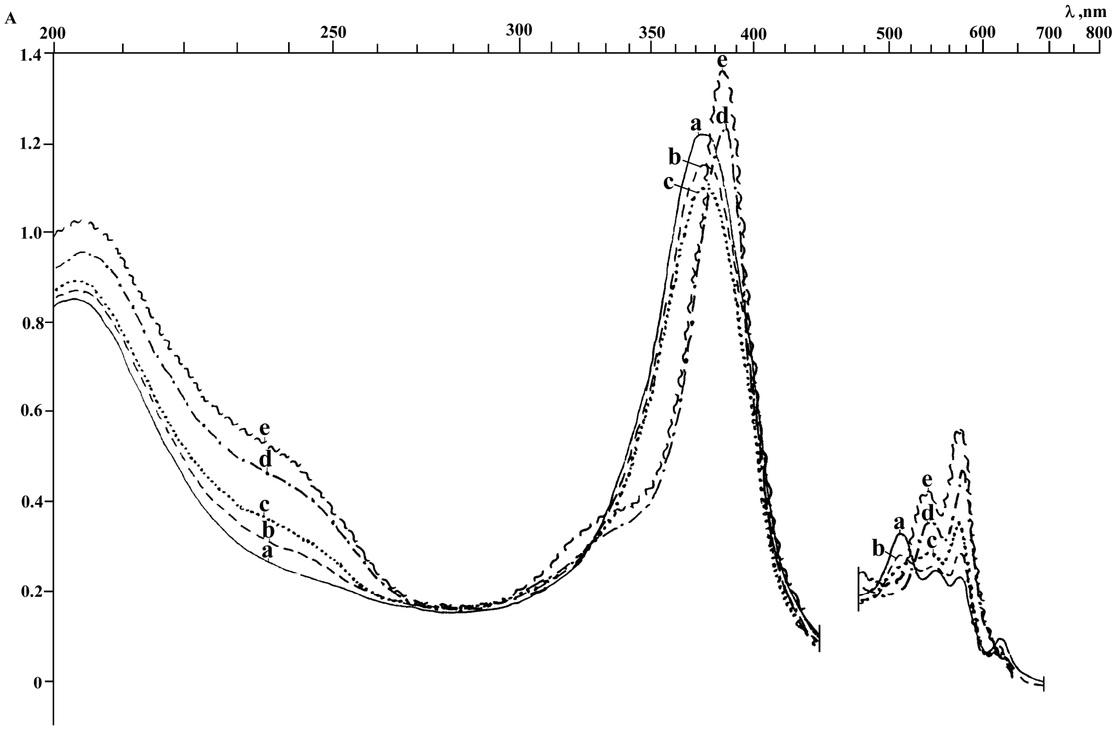

Fig. 2. Electronic absorption spectra of: a) aqueous alkaline solution of Hp; b) an hour after mixing of the ligand and $Cu(NO_3)_2$ solutions (Cu:Hp=1); c) 4 hours later; d) a day later; e) 2 days later, at the end of the reaction

In order to study its solid-state structure, the complex was isolated as a powder by adding of HNO_3. The analytical data obtained correspond to the general formula $[Cu(Hp_{-2H})].2H_2O$. The bands in the electronic absorption spectrum of the complex dissolved in KOH (Figure 3) - Soret band at 385 nm ($9.3x10^4$ $mol^{-1}.l.cm^{-1}$) and two Q bands at 553, 586 nm ($7.7x10^3$ and $9.7x10^3$ $mol^{-1}.l.cm^{-1}$) - proved its metalloporphyrin structure. In addition, the complex was paramagnetic and for the EPR investigations, magnetically diluted Cu(II)-Hp complex in diamagnetic Zn(II)-Hp matrix samples were used.

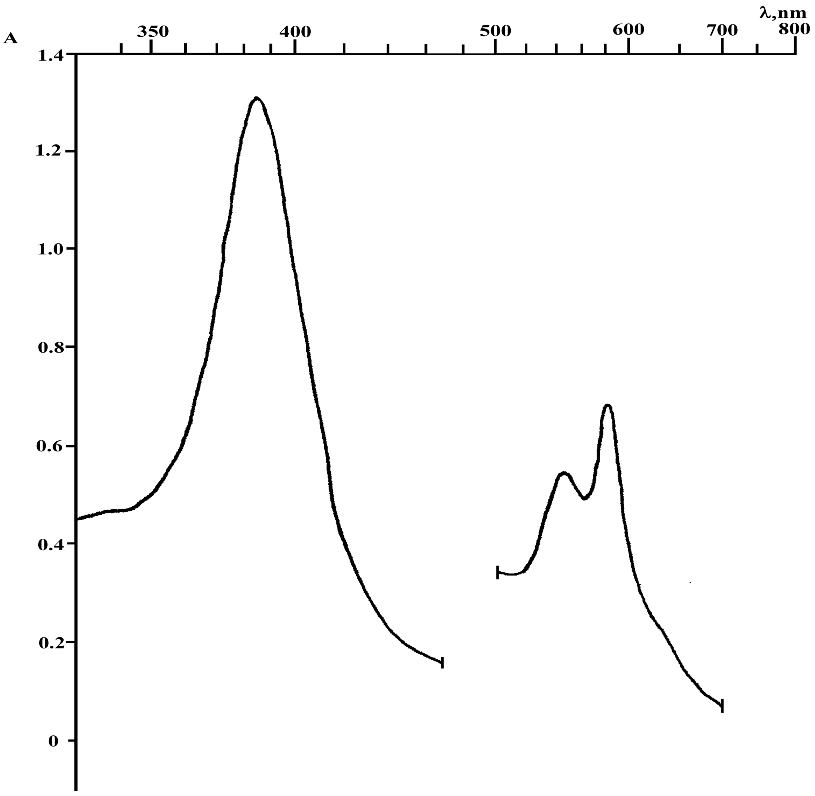

Fig. 3. Electronic absorption spectrum of the complex [Cu(Hp$_{-2H}$)].2H$_2$O dissolved in 1x10^{-1} KOH.

The powder EPR spectrum of the magnetically diluted [Cu(Hp$_{-2H}$)].2H$_2$O complex is shown on Figure 4. The principal values of the **g**-tensor, measured from the experimental spectrum, are $g_{\perp}$=2.155 and g_{II}=2.325. The hyperfine structure from the copper nuclei (I=3/2) corresponds to a tensor with axial symmetry with principal values $A_{\perp}$(Cu)=30.2x10^{-4}cm^{-1} and A_{II}(Cu)=213.8x10^{-4}cm^{-1}. An additional superhyperfine structure from ^{14}N (I=1) is observed. The principal values of the axially symmetrical superhyperfine coupling tensor are $A_{\perp}$(N)=15.1x10^{-4} cm^{-1} and A_{II}(N)=16.3x10^{-4} cm^{-1}. To obtain more precise values of all spin-Hamiltonian parameters, a fitting procedure (varying all parameters by *modified simplex method*) using simulation and comparison with the experimental EPR spectrum was used [22]. The starting values for fitting are those, measured from the experimental spectrum. For simulation model it is assumed that the copper ion is surrounded by 4 nitrogen nuclei situated on the edges of a square. Every two nitrogen nuclei in opposite edges are equivalent with axial superhyperfine coupling tensors. Calculation of the energy levels of the unpaired electron in this case shows that the energy does not depend on polar angle φ but only on angle θ. For this reason the computer time for simulation and respectively for fitting was drastically reduced. The simulated EPR spectrum in Figure 4b was obtained using the best fit parameters. The relatively good coincidence of the experimental and simulated spectra proved the square planar structure (II) for the complex, in which copper (II) is coordinated to the four pyrrole N-atoms from the porphyrin macrocycle.

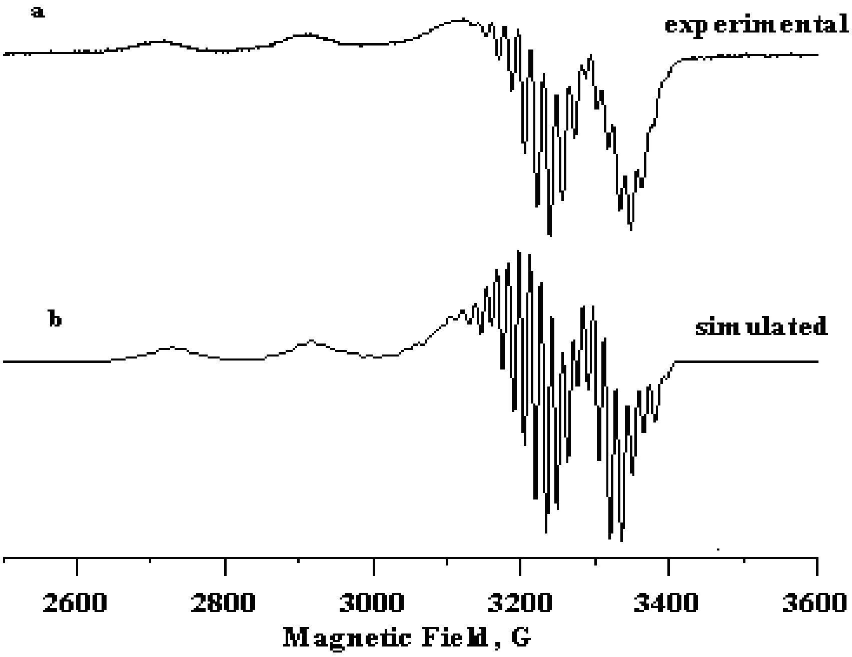

Fig. 4. EPR spectrum of the polycrystalline sample of magnetically diluted complex [Cu(Hp$_{-2H}$)].2H$_2$O: a) experimental; b) simulated.

The solid-state IR spectrum of the [Cu(Hp$_{-2H}$)].2H$_2$O complex proved the coordination of the ligand through pyrrole N atoms in the porphyrin cavity (Figure 5a). The assignments of the bands from the complex IR spectrum were made in accordance with the IR **data and NCA** published for metalloporphyrins[22].

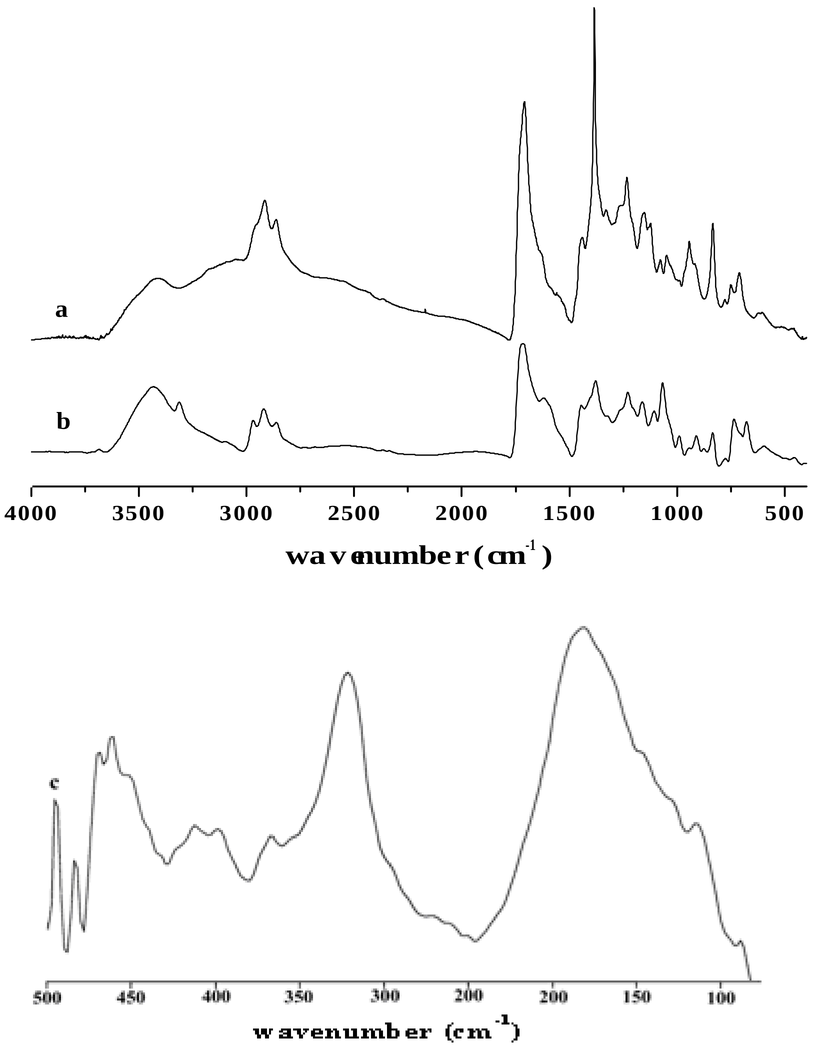

Fig. 5. Middle infrared spectra (KBr- disk): a) [Cu(Hp$_{-2H}$)].2H$_2$O; b) hematoporphyrin; c) FAR-infrared spectrum of [Cu(Hp$_{-2H}$)].2H$_2$O (CsI-disks).

The comparison of the free ligand and complex spectra (Figure 5a,b) shown identical ν(OH) and $\nu^{as,s}$(CH$_3$, CH$_2$) stretching vibrations for the hydroxyethyl, methyl, and propionic side chains. The characteristic, sharp pyrrole NH absorption at 3311 cm^{-1} together with N-H in plane and out-of-plane deformation bands from the ligand spectrum disappear in the complex spectrum and thus indicates a metal insertion into the porphyrin macrocycle. The strong absorption band of the propionate carbonyl stretch in the spectrum of the complex showed that in the solid state complex the side carboxylic groups are restored and thus eliminate the coordination out of the porphyrin macrocycle. The observed in-plane and out-of-plane skeletal vibrations in the Middle- and FAR regions (Figure 5c) correspond to the metalloporphyrin formation too. The coordination via pyrrole N-atoms is supported also by the strong absorption band at 462 cm^{-1} assigned to Cu-N stretching vibration. In addition, the presence of water in the complex follows from the bands for $\nu^{as,s}(H_2O)$ in the range 3450-3350 cm^{-1} and $\delta(H_2O)$ at 1630 - 1600 cm^{-1}.

4. Conclusion

Studying the interaction of Cu(II) and hematoporphyrin in alkaline aqueous solution at equimolar ratio on air it was established that a diamagnetic metalloporphyrin-type complex most probably of Cu(I) is the final product of the coordination reaction in solution. An intermediate sitting-atop-type complex was also recoded during the reaction course. A paramagnetic metalloporphyrins-type of Cu(II) with composition $[Cu(Hp_{-2H})].2H_2O$ was isolated from this reaction system in the solid state. The structure of the complex was solved using EPR, IR and UV-Vis spectra. Summarizing the data obtained for the interactions of hematoporphyrin with different metals as Pt(II), Pd(II), Au(III) [13 - 16] including also Cu(II) at the same reaction conditions, it could be concluded that in all reaction systems studied the complexation is accompanied with parallel redox process. According to their nature and properties, in the stable mononuclear hematoporphyrin complexes obtained the metal ions could possess unusual oxidation state as Pt(III), Pd(III) and Au(II). While in the solid state hematoporphyrin complex copper is in the oxidation state +2.

Acknowledgements

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