ORIGINAL SCIENTIFIC PAPER

CROATICA CHEMICA ACTA

Croat. Chem. Acta **2023**, *96*(1), 21–32

DOI: 10.5562/cca3950

HKD 1926

Synthesis and Study of Spectral-luminescent and Biologically Active Properties of Mixed-ligand Complexes of Cobalt(II) and Copper(II) with Rutin and Glycine

Olena Trunova,[1] Mykhailo Artamonov,[1,*] Lidia Babenko,[2] Tamara Makotryk[1]

[1] V. I. Vernadsky Institute of General and Inorganic Chemistry of the National Academy of Sciences of Ukraine, Kyiv, Ukraine
[2] M. G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine, Kyiv, Ukraine
* Corresponding author's e-mail address: artamonov.mykhailo@gmail.com

RECEIVED: December 16, 2022 ⭑ REVISED: May 11, 2023 ⭑ ACCEPTED: May 11, 2023

Abstract: Mixed-ligand complexes of Co(II) and Cu(II) with rutin and glycine were synthesized and studied by the method of "block" synthesis. The composition and structure of the synthesized compounds in solutions and in the solid state were studied, and their spectral and luminescent properties were determined. It is shown that the mixed-ligand complexes have the composition $M^{II}(Gly)_2(Rut)$, while the coordination of 3-d metal ions occurs through the catechol fragment of rutin and the carboxyl and amino groups of two glycine molecules. For complexes in solutions, luminescence was detected in the visible region of the spectrum (~500−540 nm). It is shown that 3-d metal ions quench the luminescence of rutin, but in the presence of glycinate ion, the luminescence intensity of the complexes increases. The antibacterial activity of the complexes against pathological bacteria *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *P. Aeruginosa* was studied. It has been shown that the synthesized compounds have an antimicrobial effect.

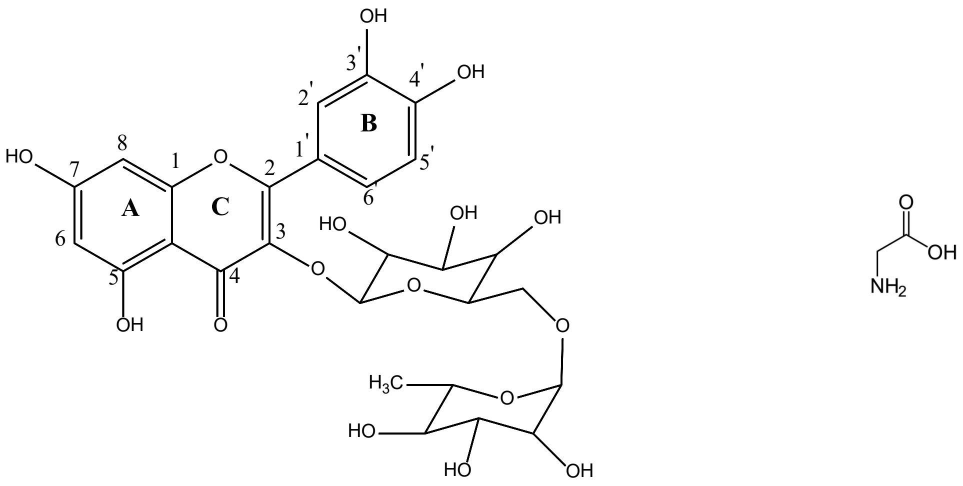
Keywords: complexes, copper, cobalt, rutin, flavonoids, luminescence.

INTRODUCTION

FLAVONOIDS are a large group of phenolic compounds that exhibit significant biological and therapeutic activity, including antioxidant, antibacterial,[1] anti-inflammatory, antidiabetic,[2] antitumor, antiallergic,[3] anti-atherosclerotic, have neuroprotective effects and more.[4–6] Among flavonoids that exhibit high pharmacological activity, the quercetin glycoside rutin (3,3', 4', 5,7-penta-hydroxyflavone-3-rhamnoglucoside $C_{27}H_{26}O_{16}H_4$, H4L, Rut) (Figure 1a) has the most pronounced therapeutic effect and widely used as components of medicines.[7,8]

Flavonoids, due to the successful arrangement of hydroxy and oxo groups in the structure of molecules, form stable chelates with various metal ions.[5,9,10] It should be noted that the biological activity of metal complexes is significantly increased compared to free ligand. Thus, N. E. A. Ikeda *et al.*demonstrated that coordination compounds of rutin significantly increase the antioxidant capacity and as a result improve other properties of the compounds (especially antitumor).[11]

One of the ways of purposeful modeling of the properties of complexes is the creation of mixed-ligand (heteroligand) complexes. In the presence of free coordination sites in monolligand complexes, usually occupied by water molecules, it is possible to attach to the central atom a certain number of other ligands to achieve the coordination saturation of the cation. Mixed-ligand complexes (MLC) play an important role in nature, because biological fluids contain a large number of biomolecules - potential ligands (vitamins, enzymes, amino acids, etc.), which can compete with complexes with biologically active metal ions (Fe, Cu, Co, Zn, Mg etc.) *in vivo*.[12] It is known from the literature that the synthesized mixed-ligand complexes show high biological activity against various pathogenic microorganisms, which is many times higher



(a) (b)

Figure 1. The structure of the Rutin (a) and glycine (b).

than monoligand complexes and free ligands.[13–17] Mixed-ligand complexes form stable chelates, which play a central role in physicochemical, biological and physiological processes, play a vital role in the metabolism and transport of trace elements in the human body and help in activating enzymes.[18,19] Heteroligand complexes of specific structure with a number of useful properties and the prospect of use as drugs can be obtained.[10,20–22]

To obtain heteroligand complexes of 3d metals among flavonoids used quercetin, naringenin, neocuproin and kaempferol, and as a second ligand - heterocyclic imines (2,2′:6′,2″ - terpiridine, 2,2′ - bipyridine)[10,17,19,20,23–27], N,N,N′,N′ - tetramethylethylenediamine,[23] 1,10-phenanthroline,[17,20,23,24,28,29] Schiff's curcumin derivatives,[30] dihalic acid,[31] aspartic acid.[32]

Biological or pharmaceutical activity was investigated for all obtained flavonoid heteroligand complexes. Thus, S.Vimalraj *et al.* showed that mixed-ligand complexes of copper with flavonoids can be used as pharmacological agents in orthopedics.[33]

It should be noted that currently there are practically no data on mixed-ligand complexes with divalent 3d transition metal ions based on rutin. We have previously studied rutin complexes with copper(II) and cobalt(II) in aqueous-ethanol solutions.[34] It is shown that in weakly acidic or neutral medium complexes Cu(II) and Co(II) with rutin are formed with the participation of 5-OH and 4-C=O fragments A and C of the ring, and in alkaline medium chelation occurs on the catechol fragment of ring B routine. In this case, the metal complexes have solvent molecules in the coordination environment, ie the coordination sphere of the metal is not completely saturated with the donor groups of the phenolate ligand. Therefore, on the basis of monometallic complexes, it is possible to obtain mixed-ligand complexes, when the donor atoms of the additional ligand will serve as elements of the completion of the structure to maximize the use of the coordination capacity of the cation. Glycine (aliphatic amino acid, Gly) (Figure 1b), which is a biologically active substance and is widely used as a drug, was chosen as an additional ligand.[35,36] The introduction of the phenolates of metals of the second biologically active ligand will increase the solubility of the complexes, increase their bioactivity due to the synergistic effect and expand their scope. We were the first to synthesize and study complex formation in ternary systems M (II) –rutin–glycine (where M(II) - Cu(II), Co(II)). Synthesis and study of the properties of mixed-ligand complexes was performed in solutions and solid state, their biological activity was studied.

EXPERIMENTAL

Materials and methods

All chemicals were analytical grade and used without further purification. Rutin and glycine were purchased from Sigma-Aldrich). $CuCl_2\cdot 2H_2O$ was purchased from Energy Chemical Company. $CoCl_2\cdot 2H_2O$ was purchased from Energy Chemical Company.

The working solutions of rutin and glycine (0.1 and 0.001 mol/dm^3) were obtained by dissolving the appropriate weighed amounts of compounds in ethanol-aqueous solutions (1 : 5), and pH = 7.5 were fixed with 0.10 and 0.01 mol/dm^3 NaOH. 0.001 mol/dm^{3}solutions of copper(II) and cobalt(II) chlorides were obtained by dissolving the appropriate weighed amounts of the salts in redistilled water. The solutions were prepared directly before synthesis.

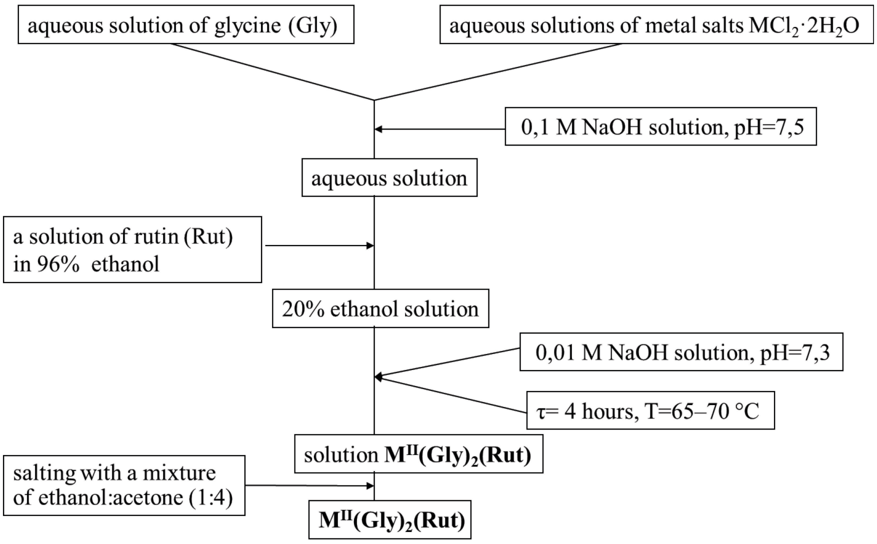


Figure 2. Synthesis of mixed-ligand complexes.

Cultures of pathological bacteria *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *P. Aeruginosa* for the study of biological activity of Cu / Co complexes based on rutin were purchased from GibcoBRL and provided by the M.G. Kholodny Institute of Botany National Academy of Sciences of Ukraine.

Electronic absorption spectra (UV-VIS spectra) were recorded on a UV/VIS Specord 210 Plus spectrophotometer (Analytik Jena AG) in quartz cuvettes with l = 1 cm (measurement range 190–1100 nm; relative error in the measurement of optical density ± 0.005).

The IR spectra were recorded by using KBr pellets in the spectral range 400–4000 cm^{-1} on a Shimadzu FT-IR 8400S instrument.

The pH value was recorded on a Mettler Toledo Seven Easy pH meter (accuracy ± 0.05) at 20 ± 3 °C and constant ionic strength μ = 0.1 M (KNO_3).

The elemental analyses for Carbon, Hydrogen and Nitrogen were obtained on microanalyses Perkins-Elmer 240c. The accuracy of measurements is 0.2–0.3 %.

The excitation and luminescence spectra of rutine solutions and its complexes (1×10^{-4} M in H_2O) were recorded on the spectrofluorimeter Fluorolog FL 3-22 (Horiba Jobin Yvon, 450 WXe-lamp) using OS 11 filter.

The synthesis of mixed-ligand complexes was performed by the "block" method, taking into account the thermodynamic constants of stability of monolithic complexes. $\lg K_{st}$ MRut and MGly complexes differ in several orders ($\lg K_{st}CuGly_2 = 15.66$, $\lg K_{st}CoGly_2 = 9.16$,[37] $\lg K_{st}CuRut = 18.51$),[34] therefore, MLCs are formed according to Scheme (Figure 2), when a complex with less stability acts as a "building block" for obtaining a multi-ligand compound by exocoordination of the second ligand.

In the first stage, equimolar amounts of aqueous solutions of metal salts and glycine were mixed, and the pH of the solution was adjusted to 7.5 with 0.1 M NaOH solution. After equilibration (~ 40 min), an ethanolic solution of rutin was added to the reaction mixture, stirred and the pH was adjusted to ≈7.3. The reaction mixture was heated for 4 hours at a temperature of 65–70 °C. After cooling, the synthesized compounds were salted out of solution with a mixture of ethanol and acetone (1:4), washed with alcohol and dried in air. The obtained MLC complexes are thermally stable (melting point about 160 °C). Elemental analysis data for the synthesized MLC complexes are given in Table 1.

RESULTS AND DISCUSSION

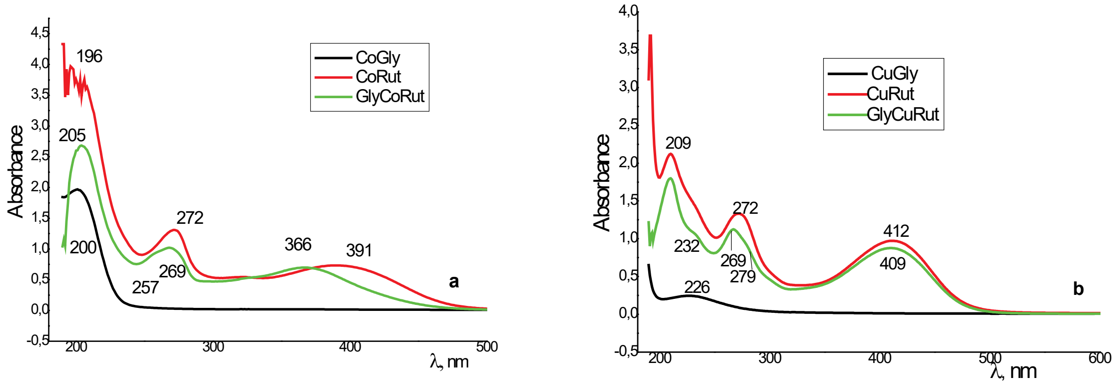
From our previous study, it was found that pH = 7.5 is optimal for the complexation of Cu(II) and Co(II) with rutin in solutions.[36]

UV/VIS study

The processes of complexation in ternary systems Cu(Co)RutGly studied by UV/VIS-method at an equimolar ratio of components and a concentration of 1×10^{-3} M. On Figure 3 shown the comparative electronic absorption spectra of monoligand complexes Cu(Co)Rut and Cu(Co)Gly with mixed-ligand complexes. In the electronic spectra of MLC solutions, absorption maxima are observed that are characteristic of the rutin molecule: the range from 320 to 385 nm is responsible for electronic transitions in the B ring, the range from 205 to 275 nm is associated with the absorption of A ring. Compared with the absorption maxima of glycinate (200 nm for Co, 226 nm for Cu) and phenolate (196; 272; 391 nm CoRut; 209; 272; 412 nm CuRut) 3-d metal

Table 1. The results of the elemental chemical analysis of mixed-ligand complexes of Cu(II), Co(II) with rutin and glycine

Elemental analysis / % (Calculated / found)			
Co(Gly)$_2$Rut (M = 813)			
C	H	N	Co
45.76/45.67	4.18 / 4.28	3.44 / 3.41	7.26 / 7.40
Cu(Gly)$_2$Rut (M = 817.5)			
C	H	N	Cu
45.50/45.27	4.16 / 4.20	3.42 / 3.41	7.77 / 7.79



(a) (b)

Figure 3. Fig. 3 UV-VIS spectra of CoGlyRut (a); CuGlyRut (b) solutions.

complexes in multi-ligand systems, the maxima of intraligand transitions of the π→π* type of rutin and glycine are shifted in the short- or long-wave region, respectively, which indicates the formation of mixed-ligand complexes.

In addition, in M^{II}GlyRut systems, the intensity of absorption decreases, which indicates a complexation. Given that the maximum absorption of M^{II}GlyRut is closer to λ_{max} of M^{II}Rut complexes, we can assume that the structure of MLC is determined by rutin, but the bonds of the central atom with Rut donor groups are weakened, which is confirmed by hypsochromic shift of the main π→π* transitions of rutin.

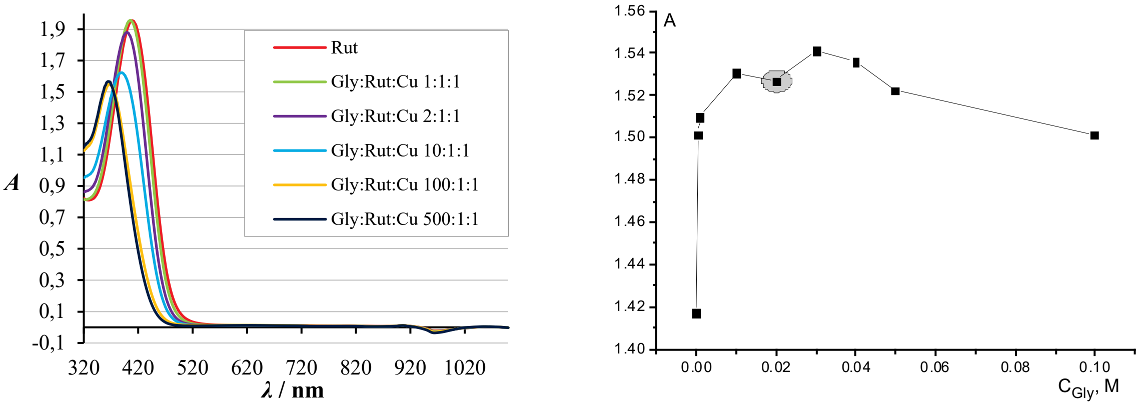
In order to obtain information on the quantitative composition of ligands included in the internal coordination sphere of MLC, the dependence of optical density on Gly concentration at constant concentrations of Rut, M (II) and pH (~ 7.3) was studied (Figure 4a, 4b).

Absorbance value A in relation to glycine concentration in both systems changes in the same way: with increasing ligand concentration from 5×10^{-5} M to 2×10^{-4} M there is an increase in absorbance and there is a well-defined inflection, which corresponds to the ratio M(II) : Gly : Rut = 1 : 2 : 1, and the composition of the complexes corresponds formula M^{II}(Gly)$_2$(Rut).

It should be noted that bands from d–d transitions inside metal orbitals are observed in the electronic absorption spectra of mixed-ligand complexes. Thus, for mono- and mixed-ligand complexes of cobalt, absorption bands in the region 515−530 nm are observed, which correspond to the spin-allowed transition from the ground state $^4T_{1g}(^4F)$ to the state $^4T_{1g}(^4P)$ (Table 2).

However, in MLC, the absorption band maxima are shifted to the long wavelength region compared to the CoRyt and CoGly monocomplexes, which indicates the passage of the process of mixed-ligand complex formation. The glycinate anion is introduced into the inner coordination sphere of the phenolate complex, and the metal–rutin bonds are weakened due to the disruption of the structure of the CoRyt monocomplex by the second ligand, which is probably due to the mutual repulsion of the ligands. The bathochromic shift of the maxima of the d–d transitions of Co^{2+} indicates a decrease in the degree of axial distortion and an increase in the acceptor properties of ligand donor atoms, i.e., strengthening of metal–ligand bonds due to the formation of an additional number of Co(II) bonds with Rut and Gly functional groups.

The electronic absorption spectra of solutions of copper complexes with the studied ligands contain broad



(a) (b)

Figure 4. UV-VIS spectra of solutions of the CuGlyRut system at different ratios of glycine at pH = 7.3 (a) and the dependence of optical density on the concentration of glycine (λ = 373 nm) (b).

Table 2. Transition energies (nm) in the UV-VIS spectra of Co(II) and Cu(II) complexes with Gly and Rut

Complex	λ_{max} / nm	Transition
CoRut	515; 531; 544	${}^4T_{1g}({}^4F) \rightarrow {}^4T_{1g}({}^4P)$
CoGly	509; 511	${}^4T_{1g}({}^4F) \rightarrow {}^4T_{1g}({}^4P)$
CoRutGly	519; 534; 543	${}^4T_{1g}({}^4F) \rightarrow {}^4T_{1g}({}^4P)$
CuRut	634; 644	$A_{1g} \rightarrow T_{2g}$
CuGly	623; 648	$A_{1g} \rightarrow T_{2g}$
CuRutGly	621; 631; 637; 643	$B_{1g} \rightarrow E_g$, $B_{1g} \rightarrow A_{2g}$, $A_{1g} \rightarrow T_{2g}$

split bands of d–d transitions that correspond to the flat-square configuration of Cu(II). However, in mixed-ligand copper-containing complexes there is a fourfold splitting of spectral bands, which may indicate a change in the coordination environment of Cu(II) due to additional interaction between copper atoms and donor atoms of glycine ligand and neighboring rutin molecules. In this case, the coordination polyhedron of copper approaches a strongly curved octahedron. The hypsochromic shift of the d–d band of the Cu^{2+} transitions may indicate an enhancement of the ligand field and, consequently, a metal – ligand bond.

Thus, for the synthesized MLC, we can propose the following structure (Figure 5).

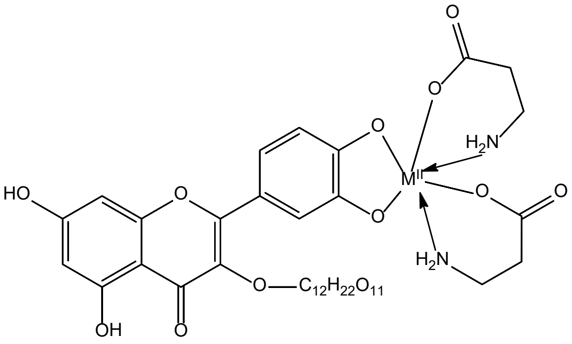


Figure 5. Proposed structure of MLC.

FT-IR spectral study

The basic vibrational frequencies and their assignment in the IR spectra of Cu(II) and Co(II) mixed-ligand complexes with rutin and glycine are given in Table 3. In a comparative analysis of the IR spectra of the synthesized MLC, the main attention was paid to the absorption bands responsible for the vibrations of the functional groups of the ligands, the donor centers of which, probably, could be associated with the metal, forming complexes.

In the IR spectra, the C=O stretching band of free rutin appears at 1660 cm^{-1}, the position of which in the MLC IR spectra undergoes a slight shift (by 4 and 7 cm^{-1}), which indicates the absence of chelation of metal ions at the carbonyl fragment routine. Obviously, this is due to the fact that the 3-OH group of the C-ring in rutin is blocked by the rutinose disaccharide. The frequency values at 1610 and 1453 cm^{-1}, corresponding to antisymmetric and symmetric stretching vibrations of the C–O bond, practically correspond to similar frequencies in the spectrum of Rut,

Table 3. Basic vibration frequencies (cm^{-1}) and their assignment in the IR spectra of mixed-ligand complexes Cu(II), Co(II) with rutin and glycine

Band assignment	Rutin	Co(II)-Rut-Gly	Cu(II)-Rut-Gly	Glycine
ν(C=O)	1660 s	1664 s	1667 s	-
ν(C–O–C)	1257 s	1240 s	1243 s	-
ν(C=C)	1611 s	1605 s	1600 s	-
ν(C–O–H)	1319 m	1361 m	1364 m	-
ν_{as}(C–O)/ν_s(C–O)	1610/1453 s	1600/1449 s	1596/1450 s	-
ν_{as}(COO$^-$)	-	1609 s	1601 s	1581 s
ν_s(COO$^-$)	-	1358 s	1361 s	1421, 1445 s
ν_{as}(NH)/ν_s(NH)	-	2975/2688 b, w	2975/2685 b, w	2955/2655 b, w
ν(M–O)	-	597, 543 w	592, 541 w	-
ν(M–N)	-	436 w	437 w	-
ν(OH)	3410 b, s	3432 b, s	3421 b, s	3400 b, s

b - broad, s - strong, m - medium, w – weak

which confirms the absence of M^{II} ions bonding with the hydroxyl group of 5-OH and the oxygen atom of the carbonyl group (4-C=O) rutin.

The bands in the IR spectrum of rutin at 1611 and 1257 cm^{-1}, related to the frequencies ν(C=C) and ν(C–O–C), are shifted in the spectra of the complexes to the low frequency region, which indicates an increase in the bond order, which is usually observed when metal coordination associated with the orthophenolic OH group of the B-ring of rutin. This conclusion is also confirmed by the shift of the ν(C–O–H) vibrations by 42 and 45 cm^{-1} in the spectra of the complexes compared to the position of this band in the spectra of the pure ligand (1319 cm^{-1}). In addition, the appearance of stretching vibrations ν(MO) in the region of ~590−540 cm^{-1} indicates the formation of metal complexes, since this band is absent in the spectrum of rutin. Based on the analysis of IR spectra, it can be assumed that in MLC, rutin is coordinated to metal ions as a bidentate ligand through the catechin fragment.

Glycine is coordinated on the other side of the plane with the same metal ion, as indicated by spectral changes in the position of stretching asymmetric and symmetric vibrations of the NH– group observed in the IR spectrum of glycine at 2955 and 2655 cm^{-1}, respectively. In the spectra of the complexes, these vibrations are bathochromically shifted by ~20 cm^{-1}, which is associated with the coordination of glycine ions to the cation through nitrogen atoms. The asymmetric ν(COO$^-$) band of free glycine at 1581 cm^{-1} is shifted in the complexes towards higher wavenumbers (1601 and 1609 cm^{-1}). In addition, the symmetric ν(COO$^-$) mode observed for glycine at 1421 cm^{-1} is shifted in the MLC IR spectra to the low frequency region by ~60 cm^{-1}, which indicates the coordination of the carboxyl group of glycine to the metal ion. The spectra of the mixed-ligand complexes contain singlet absorption bands at 436 (437) cm^{-1}, corresponding to vibrations of the M–N bond, which are absent in the spectrum of free glycine.

Investigation of Mixed-ligand Complexes of Cu(II), Co(II) with Rutin and Glycine by Luminescence Method

In the electronic absorption spectrum of an aqueous-ethanolic solution of rutin, there are two absorption bands due to intramolecular $\pi \rightarrow \pi^*$ transitions: the first band with a maximum at 360 nm corresponds to the absorption of the cinnamoyl fragment of the molecule, which is associated with the conjugate system between ring B and the carbonyl fragment of ring C. The second absorption band at 257 nm refers to $\pi \rightarrow \pi^*$ transitions in ring A of the benzoyl fragment (Figure 6). Both bands have sufficiently high molar extinction coefficients (Table 3), which indicates the effective rutin absorption of light energy. And this

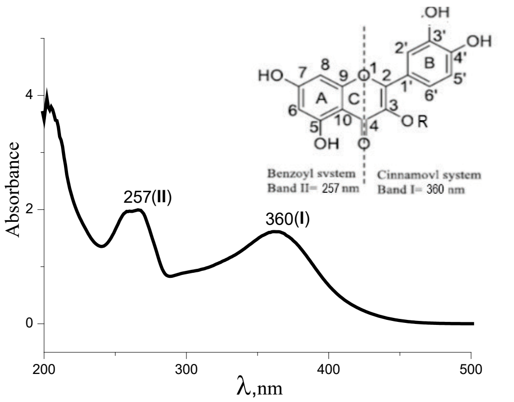
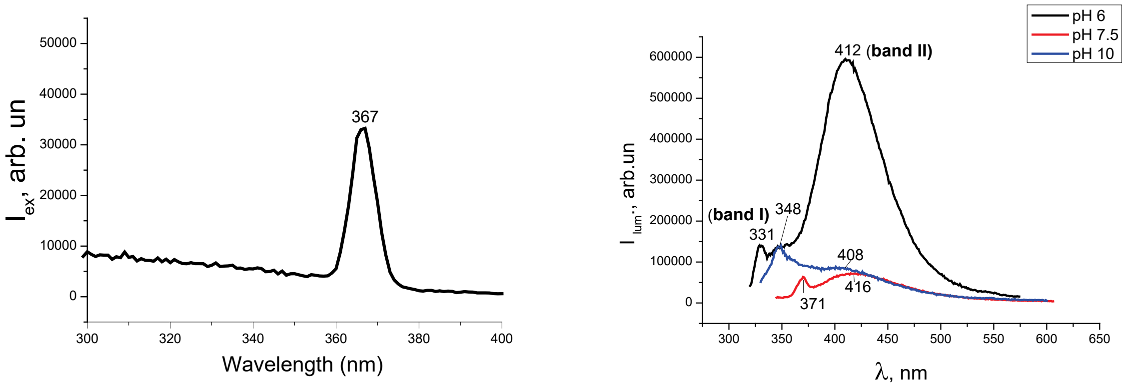


Figure 6. UV absorption spectrum of an aqueous-ethanol solution of rutin at pH = 7.5.



(a) **(b)**

Figure 7. The excitation spectra (a) and fluorescence spectra (b) of rutin.

determines its ability to exhibit luminescent properties. The excitation and fluorescence spectra of rutin of rutin at different pH values shown on Figure 7. As can be seen, rutin is not a very strong fluorescent flavonoid, which can be associated with the 5-OH group of the ring A, so in case of photoexcitation it can take part in the intramolecular proton transfer in the excited state to the neighboring carbonyl group 4C=O in the first singlet and first triplet electron excited states[38].

The spectrum of rutin radiation in ethanol consists of two bands: a weak short-wavelength band at 371 nm (pH = 7.5) and an intense band at 412 nm (pH = 6). However, with increasing pH of the solutions, the luminescence intensity of second band decreases significantly and takes the form of a structureless band, which may be associated with partial oxidation of flavonoid.

The excitation spectrum of fluorescence (Figure 7a) differs from the corresponding absorption spectrum by only 7 nm, which indicates the existence in solutions of only one conformer of rutin without changing its structure at different pHs. Luminescence is manifested due to intraligand $\pi \rightarrow \pi^*$ transitions in benzene and cinnamoyl fragments of rutin (state S_0) and effective singlet-triplet intercombination conversion. The energy gap between the lowest singlet state S_1 and the ground state S_0 is 56 nm ($\sim$18000 cm^{-1}), and the electronic radiative transitions from the state S_1 are allowed π, πn transitions. The integral intensity of short-wave radiation at pH = 6 is 14 times greater than long-wave. The first band of radiation belongs to the usual fluorescence of the Stokes shift, which does not have a large displaced fluorescence during intramolecular proton transfer, and the second - to phosphorescence.[39]

In the case of complex formation with transition metal ions (Cu, Co), the relative luminescence intensity of rutin decreases (Figure 8, Table 4), and the maximum radiation shifts to the long-wavelength region, which indicates the formation of complexes. The spectral-luminescent characteristics of rutin and its complexes are influenced by the pH of solutions (Figure 8a): the minimum value of $\lambda_{radiation}$ is observed for pH = 6, and the maximum - for pH = 7.5 (in the case of MLC) or pH = 10 (in the case of MRut), because mixed-ligand complexation shifts the pH to a more acidic region. The shift of the maximum of the radiation band of the ML complexes in comparison with the monoligand complexes at 72 (CoRut → CoRutGly) and 23 (CuRut → CuRutGly) nm indicates the coordination of glycine to the central ion. For all synthesized complexes there is a decrease in luminescence intensity (I_{lum}) compared to pure rutin.

Table 4. Spectral-luminescent characteristics of complexes

Compound	$\lambda_{absorption}$ / nm	$\varepsilon \cdot 10^4$ / l sm^{-1}·mol^{-1}	$\lambda_{radiation}$ / nm	$I_{radiation.}$·10^2 / a.u.
Rutin	257; 360	2,11	370; 410	635
CoRut	272; 391	9,87	396	395
CuRut	272; 412	10,58	520	1,3
CoRutGly	269; 366	12,11	468	510
CuRutGly	269; 409	13,14	543	37

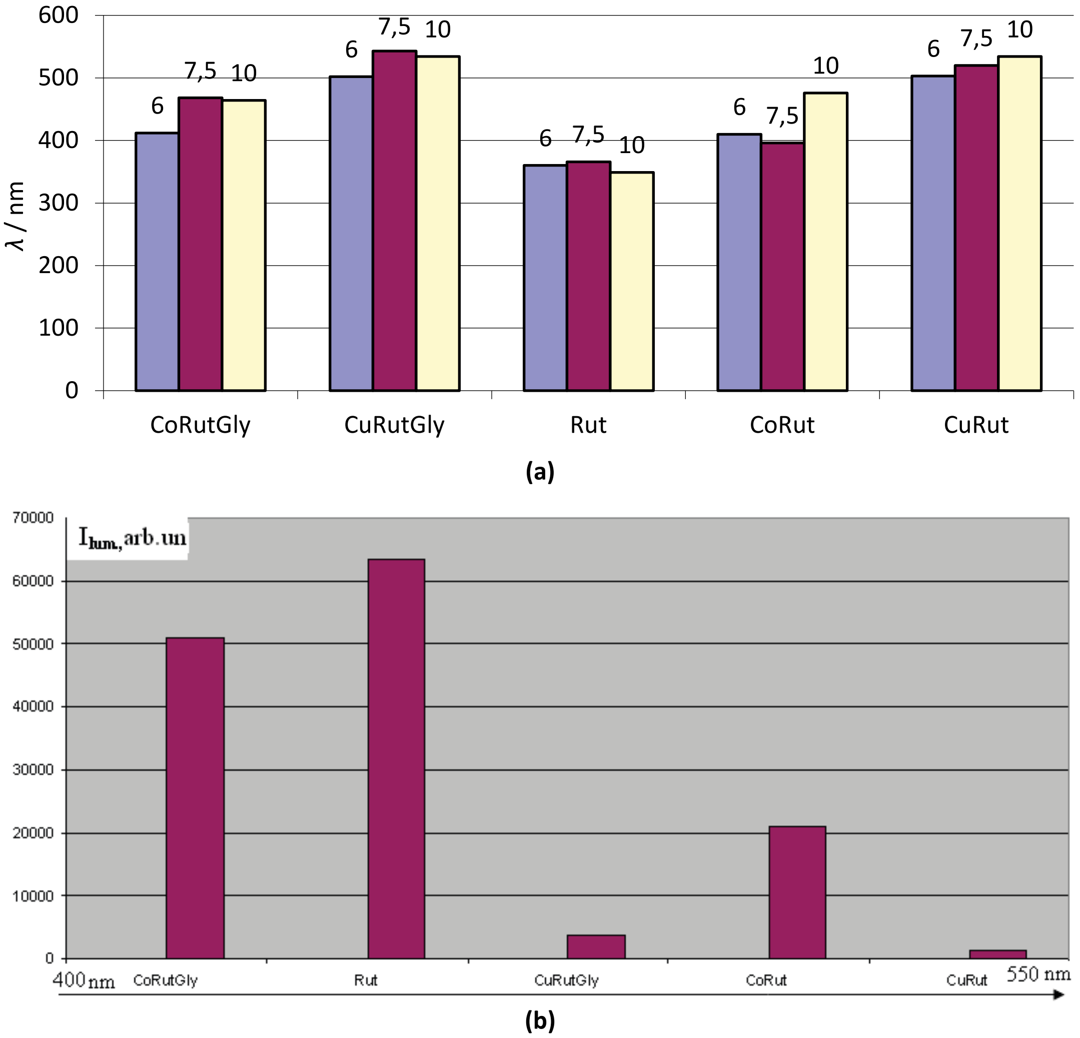


Figure 8. Dependence of the luminescence maximum on pH (a) and change in luminescence intensity (b) for complexes of 3d-metals with rutin.

Luminescence quenching in binary complexes can be associated with the dissipation of excitation energy on OH groups of water molecules (solvent), which remain in the internal coordination sphere of the cation, and by shielding emitting sublevels of the ligand by 3d-metal ions (Figure 9). That is, additional intermolecular interactions can occur in metal complexes, which affect the fluorescence of organic molecules and reduce their emission characteristics.

But in the presence of glycinate ions, the luminescence intensity of the complexes increases. It is obvious that the bidentate ligand - glycinate ion - is coordinated by M(II) ions in the carboxyl and amino groups, which leads to the displacement of water molecules (fluorescence quencher) from the inner sphere of the monoligand complex and reducing the share of non-radiative losses of excitation energy. And as a consequence, to increase the luminescence intensity. Probably, 3d-metal ions stabilize the rutin electronic system, but shield π–π* electronic transitions, which are responsible for luminescent radiation.

In addition, the increase of the luminescence intensity of ML complexes may be due to a small difference in the energies of the two ligands, which reduces the amount of non-radiative energy losses due to its efficient transfer through a small energy gap between levels.

Investigation of Antibacterial Properties of Mono- and Mixed-Ligand Complexes of 3-d Metals with Rutin.

Together with the M. G. Kholodny Institute of Botany National Academy of Sciences of Ukraine (Department of Phytohormonology, Group of senior researcher, Ph.D. Babenko L. M.) studies were conducted to detect the

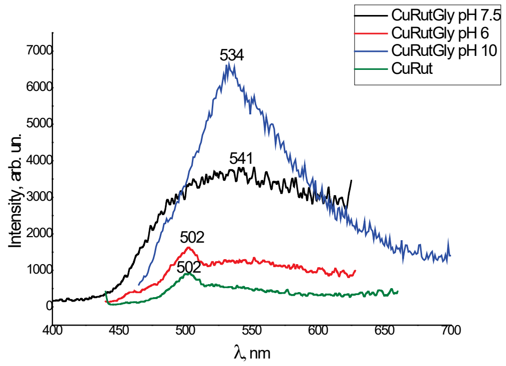


Figure 9. Luminescence spectra of rutin complexes with Cu(II) (λ = 366 nm, T = 293K).

antibacterial activity of the synthesized complexes of Co(II) and Cu(II) on the basis of rutin. Studies have been focused on pathological bacteria *Escherichia coli (Escherichia coli), Klebsiella pneumoniae, Staphylococcus aureus, P. aeruginosa* as the most rapidly developing resistance of microorganisms in modern conditions. The effect of solutions of rutin, mono- and mixed-ligand complexes of copper(II) and cobalt(II) on strains of the above cultures in 4 molar concentrations: 1×10^{-3}, 1×10^{-4}, 1×10^{-6}, 1×10^{-8} was studied. The experiment was performed in accordance with the methodology described in the article by M. Balouiri *et al.*[40]

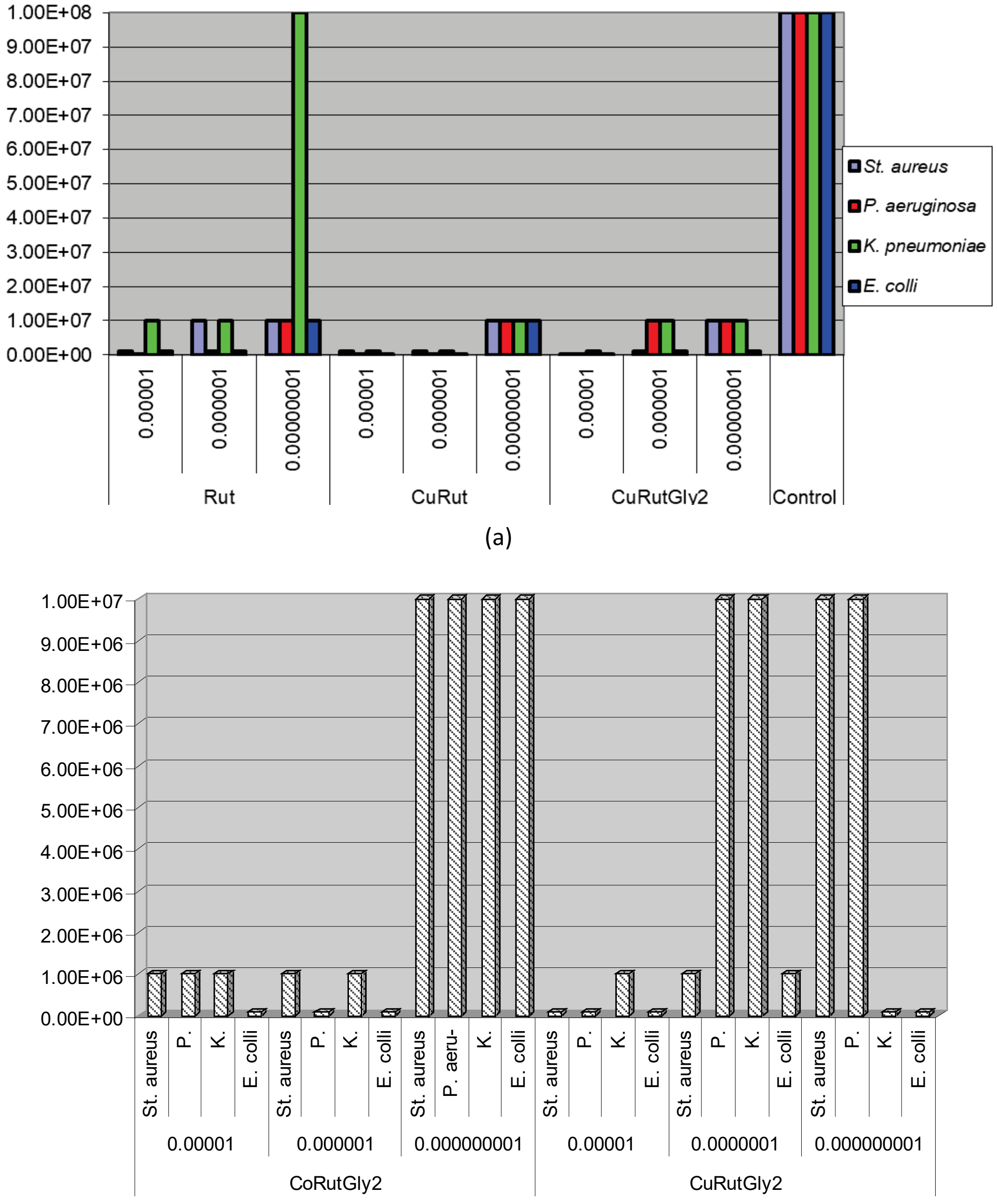
1. 24 h. culture flush of microorganisms from agar were added to LB substratum (*Lysogenybroth*), which contained 10 g of trypton, 5 g yeast extract, 10 g of NaCl, 15 g of agar, and cultured with constant stirring on a shaker for 24 hours at a temperature of 32–36 °C.
2. The resulting suspension of bacteria was diluted to the desired concentration (density 5×10^{8} CFU/ml) (CFU - colony-forming units) with sterile solutions of the studied complex and *LB* substratum. Bacterial culture in LB substratum without the addition of test complexes was used as a control.
3. Test compounds were sown on agar substratum LB on sector A of the Petri dish (20–30 strokes) using a platinum loop, 2 mm in diameter, with a capacity of 0.005 ml. After that, the loop was annealed and made 4 bar crops from sector A to sector B and similarly from sector B to C and C to D. Petri dishes were incubated at 37 °C for 24 hours, then counted the number of CFU grown in different sectors on the surface of the agar substratum. The effect of complexes on microorganisms was evaluated by the number of CFU on the surface of the agar substratum in comparison with the control.

The results obtained are given in Table 5 and in Figure 10.

The results of the study showed that the antibacterial effect of solutions of mixed-ligand complexes

Table 5. Comparative analysis of antibacterial properties of rutin and its complexes with Cu(II) and Co(II)

Compound	C / mol l^{-1}	*St. aureus*	*P. aeruginosa*	*Klebsiella pneumoniae*	*E. colli*
Rutin	10^{-3}	10^{6}	10^{5}	10^{7}	10^{6}
	10^{-4}	10^{6}	10^{5}	10^{7}	10^{7}
	10^{-6}	10^{7}	10^{6}	10^{7}	10^{6}
	10^{-8}	10^{7}	10^{7}	10^{8}	10^{7}
CoRut	10^{-3}	10^{5}	10^{4}	10^{6}	10^{4}
	10^{-4}	10^{5}	10^{5}	10^{6}	10^{4}
	10^{-6}	10^{6}	10^{6}	10^{7}	10^{6}
	10^{-8}	10^{7}	10^{7}	10^{7}	10^{7}
CuRut	10^{-3}	10^{4}	10^{5}	10^{6}	10^{5}
	10^{-4}	10^{5}	10^{5}	10^{6}	10^{5}
	10^{-6}	10^{7}	10^{7}	10^{7}	10^{6}
	10^{-8}	10^{7}	10^{7}	10^{8}	10^{7}
$CoRutGly_2$	10^{-3}	10^{4}	10^{3}	10^{6}	10^{3}
	10^{-4}	10^{5}	10^{4}	10^{6}	10^{4}
	10^{-6}	10^{6}	10^{5}	10^{7}	10^{5}
	10^{-8}	10^{7}	10^{6}	10^{7}	10^{6}
$CuRutGly_2$	10^{-3}	10^{3}	10^{4}	10^{5}	10^{4}
	10^{-4}	10^{5}	10^{5}	10^{6}	10^{5}
	10^{-6}	10^{6}	10^{7}	10^{7}	10^{6}
	10^{-8}	10^{6}	10^{7}	10^{7}	10^{6}
Control		10^{8}	10^{8}	10^{8}	10^{8}



(a)

(b)

Figure 10. Comparative diagrams of the antibacterial activity of rutin and its complexes with Cu(II) (a) and MLC Co and Cu (b).

significantly exceeds the antibacterial effect of solutions of rutin and its complexes with copper(II) and cobalt(II) and is for solutions with a molar concentration of 1×10^{-4} M: from 1×10^{5} to 1×10^{6} CFU depending on bacterial type. At the same time, this indicator is on average more than 10 times for rutin and its metal complexes, which showes their lower antibacterial activity. The greatest antibacterial activity in the experimental conditions is shown by solutions with a concentration of 1×10^{-3} M, regardless of the compound. The bioactivity of the compounds decreases with decreasing concentrations of solutions, but in general there is a tendency to increase the antibacterial activity for mixed-ligand complexes of rutin. It should be noted that copper-containing complexes are more active against pathogenic bacteria than cobalt-containing (Figure 10b).

CONCLUSIONS

Complex formation in biligand equimolar systems Cu(II) / Co(II) -glycine-rutin was studied for the first time and it was found that mixed-ligand complexes of $M^{II}(Gly)_2(Rut)$ composition are formed in solutions. It was found that in mixed-ligand complexes the coordination of 3-d metal ions occurs on the catechol fragment of rutin and carboxyl and amino groups of two glycine molecules.

The luminescent properties of Co(II) and Cu(II) complexes with rutin and glycine were studied for the first time. It has been proven that 3-d metal ions quench the luminescence of rutin, but in the presence of glycinate-ion the luminescence intensity of the complexes increases.

It has been shown that both monoligand and mixed-ligand compounds exhibit high antibacterial activity against pathological bacteria *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *P. Aeruginosa* and can be promising precursors for the development of new pharmacologically acceptable antibacterial agents in the future.

Acknowledgements. The authors are grateful to the National Academy of Sciences of Ukraine for financial support of the program "Creation of new multifunctional nanomaterials based on coordination compounds of 3d-metals and lanthanides with O, N-donor ligands", within which this work was performed. We thank also A. V. Bogatsky Physico-Chemical Institute of the National Academy of Sciences of Ukraine (Odesa, Ukraine) and in particular Senior Research Fellow Serhii Smola, for contribution in investigating by luminescence method.

REFERENCES

[1] G. Celiz, M. Daz, M. C. Audisio, *J. Appl. Microbiol.* **2011**, *111(3)*, 731–738. https://doi.org/10.1111/j.1365-2672.2011.05070.x

[2] P. Bansal, P. Paul, J. Mudgal, P. G. Nayak, S. T. Pannakal, K. I. Priyadarsini, M. K. Unnikrishnan, *Exp. Toxicol. Pathol.* **2012**, *64(6)*, 651–658. https://doi.org/10.1016/j.etp.2010.12.009

[3] M. Grazul, E. Budzisz, *Coord. Chem. Rev.* **2009**, *253(21–22)*, 2588–2598. https://doi.org/10.1016/j.ccr.2009.06.015

[4] B. Gullon, T. A. Lú-Chau, M. T. Moreira, *Trends Food Sci. Technol.* **2017**, *67*, 220–235. https://doi.org/10.1016/j.tifs.2017.07.008

[5] M. M. Kasprzak, A. Erxleben, J. Ochocki, *RSC Adv.* ***2015***, *5*, 45853–45877. https://doi.org/10.1039/C5RA05069C

[6] Yu. S. Tarakhovsky, Yu. A. Kim, B. S. Abdrasilov, E. N. Muzafarov, *Flavonoids: biochemistry, biophysics, medicine*, Synchrobook, Pushchino, **2013**, p. 5.

[7] A. Ganeshpurkar, A. K. Saluja, *Saudi Pharm J.* **2017**, *25(2)*, 149–164. https://doi.org/10.1016/j.jsps.2016.04.025

[8] S. Sharma, A. Ali, J. Ali, J. K. Sahni, S. Baboota, *Expert Opin. Invest. Drugs* **2013**, *22(8)*, 1063–1079. https://doi.org/10.1517/13543784.2013.805744

[9] H. C. Da Silva, L. A. De Souza, H. F. Dos Santos, W. B. De Almeida, *ACS Omega* **2020**, *5*, 3030–3042. https://doi.org/10.1021/acsomega.9b04174

[10] P. Alper, M. Erkisa, H. M. Genckal, S. Sahin, E. Ulukaya, F. Ari, *J. Mol. Struct.* **2019**, *1196*, 783–792. https://doi.org/10.1016/j.molstruc.2019.07.009

[11] N. E. A. Ikeda, E. M. Novak, D. A. Maria, A. S. Velosa, R. M. S. Pereira, *Chem.-Biol. Interact.* **2015**, *239*, 184–191. https://doi.org/10.1016/j.cbi.2015.06.011

[12] L. A. Finney, T. V. O'halloran, *Science* **2003**, *300*, 931–936. https://doi.org/10.1126/science.1085049

[13] J. D. Ranford, P. J. Sadler, D. A. Tocher, *Dalton Trans.*, **1993**, *22*, 3393–3399. https://doi.org/10.1039/dt9930003393

[14] S. B. Bukhari, S. Memon, M. M. Tahir, M. I. Bhanger, *J. Mol. Struct.* **2008**, *892*, 39–46. https://doi.org/10.1016/j.molstruc.2008.04.050

[15] D. Sanna, V. Ugone, A. Fadda, G. Micera, E. Garribba, *J. Inorg. Biochem.* **2016**, *161*, 18–26. https://doi.org/10.1016/j.jinorgbio.2016.04.027

[16] J. E. N. Dolatabadi, *Int. J. Biol. Macromol.* **2011**, *48*, 227–233. https://doi.org/10.1016/j.ijbiomac.2010.11.012

[17] Q. Wang, M. Huang, Y. Huang, J. S. Zhang, G. F. Zhou, R. Q. Zeng, X. B. Yang, *Med. Chem. Res.* **2014**, *23*, 2659–2666. https://doi.org/10.1007/s00044-013-0863-2

[18] G. Wu, Y. Fang, S. Yang, J. Lupton, N. Turner, *J. Nutr.* **2004**, *134(3)*, 489–492. https://doi.org/10.1093/jn/134.3.489

[19] B. S. Mendiguchia, I. Aiello, A. Crispini, *Dalton Trans.* **2015**, *44*, 9321–9334. https://doi.org/10.1039/C5DT00817D

[20] H. M. Gençkal, M. Erkisa, P. Alper, S. Sahin, E. Ulukaya, F. Ari, *J. Biol. Inorg. Chem.* **2020**, *25(1)*, 161–177. https://doi.org/10.1007/s00775-019-01749-z

[21] S. Selvaraj, S. Krishnaswamy, V. Devashya, S. Sethuraman, U. M. Krishnan, *Med. Res. Rev.* **2014**, *34*, 677–702. https://doi.org/10.1002/med.21301

[22] D. Ravishankar, A. K. Rajora, F. Greco, H. M. I. Osborn, *Int. J. Biochem. Cell Biol.* **2013**, *45*, 2821–2831. https://doi.org/10.1016/j.biocel.2013.10.004

[23] E. Balogh-Hergovich, J. Kaizer, J. Pap, G. Speier, G. Huttner, L. Zsolnai, *Eur. J. Inorg. Chem.* **2002**, *9*, 2287–2295. https://doi.org/10.1002/1099-0682(200209)2002:9%3C2287::AID-EJIC2287%3E3.0.CO;2-3

[24] A. A. Andelescu, C. Cretu, V. Sasca, S. Marinescu, L. Cseh, O. Costisor, E. I. Szerb, *Polyhedron* **2018**, *147*, 120–125. https://doi.org/10.1016/j.poly.2018.03.016

[25] J. C. C. Filho, A. L. F. Sarria, A. B. Becceneri, A. M. Fuzer, J. R. Batalhão, C. M. da Silva, R. M. Carlos, P. C. Vieira, J. B. Fernandes, M. R. Cominetti, *PLoS ONE* **2014**, *9(9)*, e107058. https://doi.org/10.1371/journal.pone.0107058

[26] Y. N. Zhang, H. M. Yin, Y. Zhang, D. J. Zhang, X. Su, H. X. Kuang, *J. Mol. Struct.* **2017**, *1130*, 199–207. https://doi.org/10.1016/j.molstruc.2016.10.034

[27] B. Xiao, H. Wang, X. Zhao, Y. Li, *Anal. Methods* **2014**, *6*, 2894–2899. https://doi.org/10.1039/c4ay00066h

[28] L. V. Tamayo, L. R. Gouvea, A. C. Sousa, R. M. Albuquerque, S. F. Teixeira, R. A. de Azevedo, S. R. Louro, A. K. Ferreira, H. Beraldo, *Biometals* **2016**, *29*, 39–52. https://doi.org/10.1007/s10534-015-9894-0

[29] R. M. M. Oliveira, J. F. Souza Daniel, R. M. Carlos, *J. Mol. Struct.* **2013**, *1031*, 269–274. https://doi.org/10.1016/j.molstruc.2012.09.066

[30] J. Porkodi, N. Raman, *Appl Organometal Chem*. **2017**, *32(2)*, e4030. https://doi.org/10.1002/aoc.4030

[31] G. Erdogan, R. Karadag, *Rev. Anal. Chem.* **2006**, *25*, 257–270. https://doi.org/10.1515/REVAC.2006.25.4.257

[32] N. Mahar, A. A. Hulio, Q. K. Panhwar, H. I. Afridil, I. Mahar, S. A. Mallah, *Med Chem (Los Angeles)* **2018**, *8(9)*, 253–258. https://doi.org/10.4172/2161-0444.1000521

[33] S.Vimalraj, S. Rajalakshmi, D. R. Preeth, S. V. Kumar, T. Deepak, V. Gopinath, S. Chatterjee, *Mater. Sci. Eng. C. Mater. Biol. Appl.* **2018***,* *83*, 187–194. https://doi.org/10.1016/j.msec.2017.09.005

[34] O. K. Trunova, M. S. Artamonov, T. O. Makotryk, *Ukr. Khim. Zh.* **2021**, *81*, 90–102. https://doi.org/10.33609/2708-129X.87.10.2021.90-102

[35] O. A. Minjaeva, *Scientific Review. Biological Sciences* **2016**, *6*, 43–47.

[36] R. Y. Gundersen, P. Vaagenes, T. Breivik, F. Fonnum, P. K. Opstad, *Acta Anaesthesiol Scand.* **2005**, *49(8)*, 1108–1116. https://doi.org/10.1111/j.1399-6576.2005.00786.x

[37] A. Casale, A. De Robertis, C. De Stefano, A. Gianguzza, G. Patanè, C. Rigano, S. Sammartano, *Thermochim. Acta* **1995**, *255*, 109–141. https://doi.org/10.1016/0040-6031(94)02181-M

[38] Y. Norikane, H. Itoh, T. Arai, *J. Photochem. and Photobiology A: Chemistry* **2004**, *161(2–3)*, 163–168. https://doi.org/10.1016/S1010-6030(03)00285-5

[39] T. Sasikumar, M. Ilanchelian, *J. of Luminescence* **2021**, *36(2)*, 326–335. https://doi.org/10.1002/bio.3945

[40] M. Balouiri, M. Sadiki, S. K. Ibnsouda, *J. Pharm. Anal.* **2016**, *6*, 71–79. https://doi.org/10.1016/j.jpha.2015.11.005