COMPLEX FORMATION BETWEEN TRANSITION METALS AND 2-PYRROLIDONE-5-HYDROXAMIC ACID

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Abstract

Complexes of cobalt(II), nickel(II) and copper(II) with 2-pyrrolidone-5-hydroxamic acid (HL) have been investigated by the combined pH-spectral titration method at 25±0.1 °C in aqueous medium of 0.2 M ionic strength. Cobalt(II) and nickel(II) form $[ML]^+$, $[ML_2]$ and $[ML_2H_{-1}]^-$ complexes with HL, while in the case of copper(II) mononuclear $[CuL]^+$ and dinuclear $[Cu_2L_2H_{-2}]$ species are present. The formation constants obtained and the possible binding modes for the complexes in aqueous solution are discussed. ^1H NMR, IR and EPR measurements have furnished additional information on the nature of binding in the complexes formed.

Introduction

The biological importance of hydroxamic acids is well established.[1] They are known as constituents of antibiotics, growth factors, food additives, tumour inhibitors and cell division factors. Hydroxamic acids have been shown to possess diverse biological activities, many of which are due to their complexing properties towards transition metal ions.[2-4] Iron(III) complexes of naturally occurring hydroxamic acids, called siderophores, are involved in the processes of iron transport from the environment into the living organisms.[5,6] Natural trihydroxamic acid, desferrioxamine B, has been widely used in the treatment of iron overload associated with β-thalassemia (Cooley's anaemia).[7] The same compound has also been employed in chelation therapy to remove aluminium from patients suffering from dialysis, encephalopathy, and Alzheimer's disease.[8] Data have also been published on the inhibitory activity of hydroxamic acid derivatives of amino acids and peptides on metalloproteinases.[9,10] The mechanism of inhibition appears to involve chelation of metals at their active sites. Some aminohydroxamic acids have been investigated with the aim of designing metal chelates as suitable sources of various trace elements essential in animal nutrition.[11]

With regard to the strong ability of hydroxamic acids to form chelates, clarification of their interactions with metal ions is of particular importance in terms of biological effects. In the present work, an equilibrium and structural studies have been performed on the cobalt(II), nickel(II) and copper(II) complexes of the 2-pyrrolidone-5-hydroxamic acid. Moreover, the preliminary results indicate that 2-pyrrolidone-5-hydroxamic acid increases Na^+/K^+-ATPase activity in the cortex and medulla of rat's kidney.[12]

Results and discussion

Acid-base properties of 2-pyrrolidone-5-hydroxamic acid

The fully protonated form of 2-pyrrolidone-5-hydroxamic acid (HL) is shown in Scheme 1. The ligand can release only one proton in the pH range 1.5-12.4, which may be attributed to the hydroxamic acid group. The determined proton dissociation constant at I=0.2 (KCl) is 8.60.
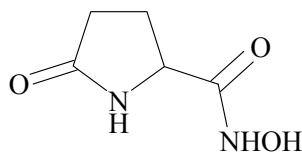
Scheme 1. Structural formula of 2-pyrrolidone-5-hydroxamic acid.

Comparison of this data with the analogous values of benzhydroxamic acid, pK_a=8.79, and acethydroxamic acid, pK_a=9.37,[13] shows an increase of acidity in the sequence acethydroxamic acid < benzhydroxamic acid < HL. This is in accordance with the fact that the pyrrolidone ring has a lower electron-donating ability than the phenyl and the methyl groups. Comparison of the obtained ionization constant with the corresponding pK values for a series α-aminohydroxamic acids shows that the presence of the $-NH_3^+$ group in aminohydroxamic acids remarkably increases the acidic character of the OH group.[14] This is a consequence of the weaker electron-withdrawing effect of the imine group in the pyrrolidone ring as compared to the $-NH_3^+$ group.

Cobalt(II)-2-pyrrolidone-5-hydroxamic acid system

Values of the stability constants obtained from the SPECFIT analysis[15,16] of the spectrophotometric pH titration data are presented in Table 1. The pK_a value for HL and

the values of the metal ion hydrolysis constants were used as fixed parameters when calculating the stability constants for investigated complexes.[17] The best fit was obtained using a model postulating formation of the following complexes: $[CoL]^+$, $[CoL_2]$ and $[CoL_2H_{-1}]^-$.

Table 1. Stability constants for complexes present in the metal ion – 2-pyrrolidone-5-hydroxamic acid systems at 25 °C and *I*=0.2 M (KCl). Standard deviations are given in parentheses.

Metal ion	Complex	$\log \beta$
Co(II)	$[ML]^+$	4.62(2)
	$[ML_2]$	8.09(4)
	$[ML_2H_{-1}]^-$	-1.75(5)
Ni(II)	$[ML]^+$	4.71(2)
	$[ML_2]$	8.11(3)
	$[ML_2H_{-1}]^-$	-1.33(5)
Cu(II)	$[ML]^+$	7.52(2)
	$[M_2L_2H_{-2}]$	10.53(9)
Fe(III)[a]	$[ML]^{2+}$	10.14
	$[ML_2]^+$	18.85
	$[ML_3]$	26.16

[a] From ref. 21

Distribution of the complex species in solution, as a function of pH, is shown in Figure 1. Complexation begins at pH values of *ca*. 4 with the formation of species $[CoL]^+$, corresponding to the displacement of one proton. Complex $[CoL]^+$ reaches the maximum concentration of 67% total cobalt(II) at pH 6.0. At pH 8, the predominant species is $[CoL_2]$. Increasing the pH up to *ca*. 8 in this system causes a new base consumption. The proton released by the $[CoL_2]$ complex leads to the formation of the $[CoL_2H_{-1}]^-$ species. This process results either from the formation of a mixed hydroxo-hydroxamate species or from the deprotonation of the bound hydroxamic group in the $[CoL_2]$ species.
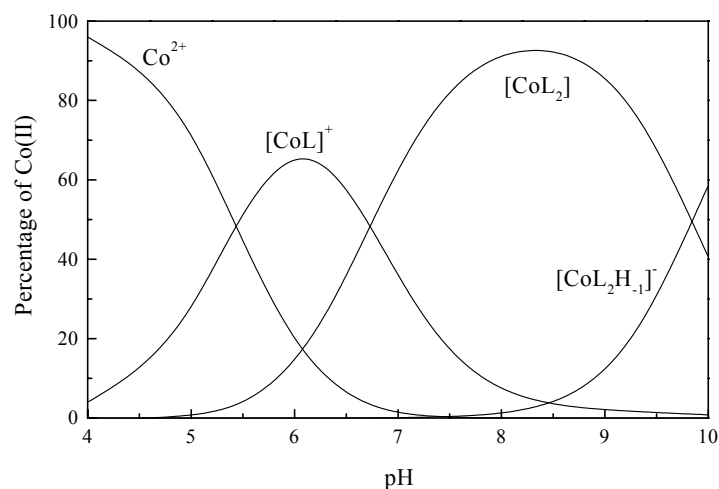
Figure 1. Concentration distribution curves for the complexes formed in the cobalt(II) –2-pyrrolidone-5-hydroxamic acid system as a function of pH at 25 ºC. [Co(II)]=0.01 M, [HL]=0.05 M, *I*=0.2 M (KCl).

To obtain more information about the bonding modes in the complexes formed, ^{1}H NMR measurements were carried out. The line broadening in ^{1}H NMR spectra is similar for all protons, at pH 7.5 where the major species is [CoL$_2$]. The difference in ^{1}H NMR chemical shifts between the complex and the free ligand is only *ca*. 0.05 ppm. Previous research on some metal aminohydroxamate showed that chelation through hydroxamate oxygens does not cause any measurable chemical shift change of the α-CH proton signals.[18] Taking this into account, the obtained results suggest that, under the conditions applied, chelation occurs *via* hydroxamate oxygens. The same conclusion can be drawn from the IR spectra in D$_2$O at pH 7.5. Electron withdrawal from the carbonyl group due to the complexation increases electron density in the C-N bond, resulting in a lowering of 32 cm^{-1} in the carbonyl frequency compared to the free ligand, and an increase of 20 cm^{-1} in the C-N frequency (Table 2).

Table 2. Selected infrared data (cm^{-1}) of ligand and complexes in D$_2$O at pH 7.5

Compound	ν(C=O) hydroxam.	ν(C=O) pyrrolidone	ν(C-N)
HL	1648	1668	1520
CoL$_2$	1616	1662	1540
NiL$_2$	1626	1686	1535

Nickel(II)-2-pyrrolidone-5-hydroxamic acid system

The obtained spectrophotometric data demonstrate that nickel(II) ion is coordinated by HL forming mono [NiL]$^+$, bis [NiL$_2$], and [NiL$_2$H$_{-1}$]$^-$ mononuclear chelates. The calculated stability constants are shown in Table 1. With increasing pH, the colour changed from green in acidic solution to yellow in the neutral region and red in alkaline media. The characteristic spectral change associated with the titration of the reactants with potassium hydroxide is shown in Figure 2. The solution electronic spectra below pH 7 exhibit two bands, at 394 nm and 680-700 nm. The [NiL]$^+$ complex is the dominant species in this pH range, reaching a maximum concentration of 77% total nickel(II) at pH 6. Increasing the pH, the spectra show the appearance of a new maximum at 518 nm. As the pH increases, a simultaneous decrease in the absorption occurs at the 680 nm maximum and an increase in the absorption at the 518 nm maximum. The dominant species at pH 7-9 is the [NiL$_2$] complex. Above pH 9.5, the λ_{max} is not changed in accordance with the formation of the [NiL$_2$H$_{-1}$] species that predominates in this range.
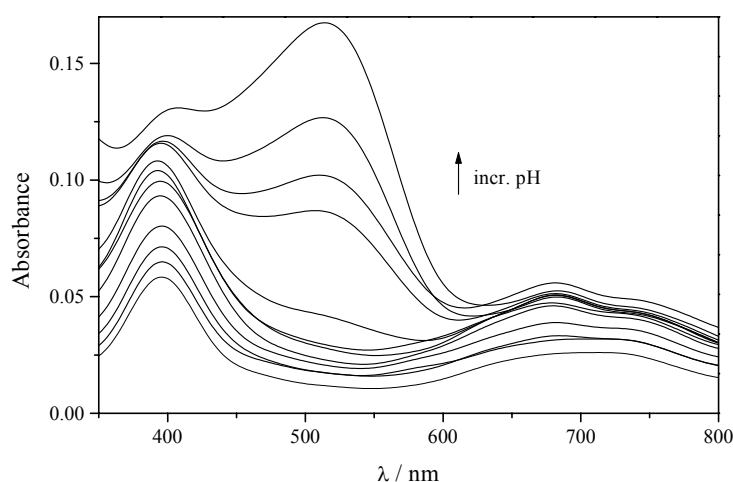
Figure 2. Absorbance spectra of the nickel(II) – 2-pyrrolidone-5-hydroxamic acid system at various pH values. [Ni(II)]=0.01 M, [HL]=0.05 M, *I*=0.2 M (KCl), *T*=25 ºC, pH=4.53-9.10.

Due to the nature of the nickel(II) ion, one would expect some preference for the formation of an (N,N)-chelate. Various spectroscopic studies of the nickel(II)-2-pyrrolidone-5-hydroxamic acid system give further support to coordination modes. The

pH dependence of the absorption spectra, in agreement with earlier observations, suggests that the geometry of the species formed below pH 7 is octahedral.[11] Comparison of the spectral characteristics of the nickel(II)-acetohydroxamic acid system (λ_{max} 389 and 671 nm) with those of [NiL]$^{+}$ indicates the same type of bonding through the two oxygen donors. Above pH 7, the formation of bis complexes with planar geometry can be assumed. The ^{1}H NMR spectra of the solution at pH 7.5 consist of resonances with the chemical shifts very close to those observed for the free-metal ligand. The chemical shift of the proton at C-5, bound to the nitrogen in the pyrrolidone ring, is not changed at this pH (4.135 ppm in the ligand and 4.130 ppm in the complex). In agreement with these findings, our results suggest that the nitrogen of the pyrrolidone ring takes no part in the coordination. The [NiL$_2$] species binding sites dominant at this pH seem to be the hydroxamic group oxygen atoms. The same coordination mode can be assumed in [NiL$_2$H$_{-1}$] species, since the ^{1}H NMR spectra of the deprotonated ligand at pH 10 are not considerably changed by the metal ion presence. The general pattern of the infrared spectra supports coordination *via* hydroxamate oxygens (Table 2). The stretching band of hydroxamic C=O in the complex is shifted by 22 cm^{-1} to a lower energy than that in the free ligand in aqueous (D$_2$O) solution at pH 7.5. There is shift of 18 cm^{-1} in the frequency of the pyrrolidone C=O group to a higher energy. The most likely explanation of this observation lies in the formation of strong intramolecular hydrogen bonding between ligand molecules.

Cooper(II)-2-pyrrolidone-5-hydroxamic acid system

The measurable pH range is quite narrow in the system containing HL and copper(II). Complex formation starts even below pH 1.8 and a precipitate appears at *ca* pH 3.5. This pH is practically the same for different metal-ligand ratios. Precipitation occurs even at a metal-ligand ratio of 10:1. The solution was pale yellow and turned green at about pH 2.5. Absorption spectra at different pH values show two bands, one at 370 nm and the other at 770 nm. Increases in absorbances and small shifts to shorter wavelengths with increasing pH were observed. The initial reaction involves one-proton displacement to form a mononuclear complex. The band with λ_{max} = 370 nm can be attributed to a hydroxamic oxygen to Cu(II) charge transfer transition. Spectroscopic

data show that the *d-d* transition energy is centred at around 770 nm, while the copper(II) aquaion absorbs at 840 nm. The shift towards higher energy clearly indicates substitution of water-bound oxygens by the stronger hydroxamic oxygen donors. In order to gain more insight into the type of coordination involved in the mononuclear complex, EPR spectra were obtained for the solution at pH 3 where species [CuL]$^+$ exhibits the highest concentration. The recorded spectra consist of four lines due to copper coupling (Figure 3). At this pH, the $g_\parallel$ (2.293) and $A_\parallel$ (145×10^{-4} cm^{-1}) spectral parameters correspond to the coordination of two oxygen atoms (O,O) and indicate that the interaction between copper(II) and HL starts *via* hydroxamate oxygens.[19] Additional support for this coordination mode comes from the comparison of the stability constants of this complex with the respective complex of acetohydroxamic acid ligand (log β_{110}=7.89), which are very similar. For the latter case, only the (O,O) donor set may be involved in the metal ion coordination.
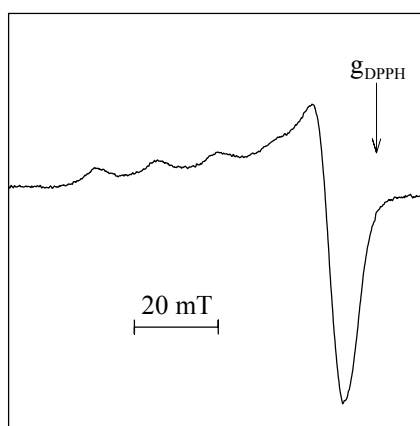
Figure 3. EPR spectra of the copper(II) – 2-pyrrolidone-5-hydroxamic acid in aqueous solution at room temperature. Conditions: [Cu(II)]=0.01 M, [HL]=0.05 M, pH=3.0.

When the reaction solution pH was increased to over 4, a dark green complex was precipitated. The precipitation occurs in the pH region where polynuclear complexes are expected to be formed because the results obtained in previous studies of copper(II)-aminohydroxamic acid systems generally confirm the formation of dinuclear species in acidic media.[20] Stability constants of only two species were determined in a limited pH

range (Table 1). The experimental data best fit a model including the formation of mononuclear $[CuL]^+$ and dinuclear $[Cu_2L_2H_{-2}]$ complexes (Figure 4). The species distribution curve indicates that formation of the dinuclear complex starts at pH 2.8. The precipitate was dissolved gradually until the pH was approximately 10, so no experimental data could be obtained after dissolution. It is obvious that formation of a bis-complex is hindered in the presence of the dinuclear complex.
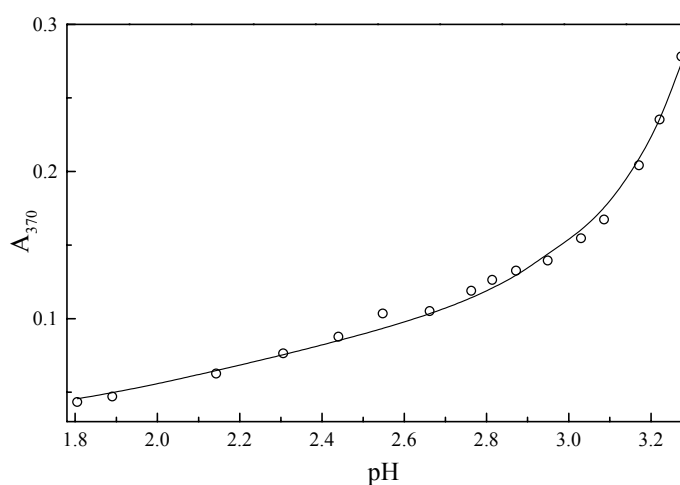
Figure 4. Dependence of the absorbance at 370 nm of the copper(II) – 2-pyrrolidone-5-hydroxamic acid on pH of solution. The solid line represents fit of the data to the stability constants given in Table 1. Conditions: [Cu(II)]=0.01 M, [HL]=0.05 M, I=0.2 M (KCl), T=25 ºC, pH=1.85-3.29.

To check on this dimer formation, in addition to spectrophotometric studies, EPR measurements were carried out as well. The apparent loss of EPR signals strongly indicates the formation of dinuclear EPR-silent species.[19] Elemental analysis of the isolated complex showed 1:1 metal to ligand stoichiometry, which is in agreement with both the mono and dinuclear species composition. However, the mononuclear complex $[CuL]^+$ requests the existence of an anion to compensate for the positive charge which is not found. This indicates that the isolated complex is of dimeric nature. The infrared spectra of this complex reveal a lowering of 20 cm^{-1} in the carbonyl frequency compared to the free ligand in solid state, and an increase of 35 cm^{-1} in the C-N frequency. The band at 3420 cm^{-1} assigned to the N-H stretching mode is also shifted to a lower frequency by 130 cm^{-1} compared to the free-ligand band, pointing to possible structural differences. Hence, these IR results suggest that the coordination of Cu(II) in the

dinuclear complex occurs by the oxygen atoms of the hydroxamate group of one molecule and by the nitrogen atoms from the deprotonated NOH⁻ group and the imine group of the pyrrolidone ring of the other molecule.

Conclusions

When the refined stability constants of the previously reported iron(III) complexes are compared with the corresponding data, it can be seen that the complexes with iron(III) are more stable and that the differences in the formation constants are of several log units.[21] Experimental data indicate that the hydroxamate oxygen atoms are involved in the coordination to the metal in all of these complexes, with the exception of the dinuclear complex of the cooper(II) where the mixed bonding mode (N,O) was proposed. The carbonyl and the imine groups of the pyrrolidone ring stay free for eventual hydrogen binding interactions with active sites of metalloenzymes.

Experimental

Materials

2-Pyrrolidone-5-hydroxamic acid was prepared *via* the dimethyl ester of glutamic acid by the method described previously[22] and was characterized by NMR and IR spectra and elemental (C,H,N) analyses. Metal-ion solutions were prepared from the metal chlorides (Sigma) by dissolving an appropriate amount in doubly distilled water or in hydrochloric acid of known concentration. Concentrations of the metal-ion stock solutions were determined by complexometric titration with EDTA.[23] All other chemicals were of analytical grade and were used without further purification. Doubly distilled and deionized water in an all-glass apparatus was used throughout the experiments.

Potentiometric Measurements

For samples used in pH-metric and spectrophotometric measurements, the ionic strength was adjusted to 0.2 M with KCl. Titrations were carried out with carbonate-free KOH solutions of known concentrations (*ca*. 0.1 M) under a purified argon atmosphere. The pH measurements were performed in a water-jacketed cell maintained at 25±0.1 °C by an external circulation bath. A Radiometer PHM 85 digital pH meter equipped with a combined glass electrode (Radiometer PHC 2406L) was used. The electrode was

calibrated in terms of [H^+] by titration of standard HCl with standard KOH solution at 25.0 °C and I = 0.2 (KCl). The SUPERQUAD computer program was used for refinement of the pK_a value from the titration data.[24]

Spectrophotometric measurements

The investigation was carried out as a series of spectrophotometric pH titrations at 25±0.1 °C. Absorption spectra were recorded on a Hewlett-Packard 8452A diode-array spectrophotometer. Concentrations of metal ions in solutions used in the spectroscopic measurements were 0.01 M. The metal-ligand ratios were adjusted in the range 1:1-1:5. The data reduction analysis of the recorded spectral data points was done by the commercially available program SPECFIT, version 2.09.[15,16]

Other measurements

The ^{1}H NMR spectra were measured on a Varian Gemini 300 spectrometer in D_2O. The concentration of the ligand was 0.14 M in all samples, while the metal:ligand ratio was 1:4. EPR spectra were recorded on a Varian E-106 spectrometer at room temperature. The concentrations were the same as those applied in the spectrophotometric pH titrations. The IR spectra were recorded with a FT-IR Perkin-Elmer Paragon 500 spectrometer in D_2O and from samples dispersed in KBr pellets. The IR measurements in D_2O were carried out at 0.1 M ligand concentrations using ZnSe cells. The metals to ligand molar ratios were adjusted to 1:2. Elemental analysis was performed on a Perkin-Elmer CHNS/O analyser 2400. A Perkin-Elmer 305B Atomic Absorption Spectrophotometer was used for copper analysis.

Preparation of copper(II) dinuclear complex

2-Pyrrolidone-5-hydroxamic acid (144 mg, 1 mmol) was dissolved in 50 mL water. To this solution, $CuCl_2$ (85 mg, 0.5 mmol) dissolved in 10 ml water was slowly added under constant stirring. Then the pH was raised to pH 3.5 by dropwise addition of 1.0 M KOH. To this solution, saturated KCl was added until precipitation occurred. The obtained product was allowed to stand overnight. The dark green precipitate was filtered off, washed with water and dried in air and finally *in vacuo* over P_4O_{10} at room temperature for 72 h. *Anal.* Calculated for $C_{10}H_{12}N_4O_6Cu_2 \cdot 5H_2O$: Cu, 25.4; C, 24.0; N, 11.2; H 4.4%. Found: Cu, 25.1; C, 24.6; N, 12.1; H, 3.5%.

Acknowledgements

References

1. Kehl, H. *Chemistry and Biology of Hydroxamic Acids;* Karger, New York, 1982.
2. Raymond, K. N. *Coord. Chem. Rev.* **1990**, *105*, 135–153.
3. Crumbliss, A. L. *Handbook of Microbial Iron Chelate;* Ed. G. Winkelmann, CRC Press, New York, 1991.
4. Raymond, K. N. *Pure Appl. Chem.* **1994**, *66*, 773–781.
5. Albrecht-Gary, A.-M.; Crumbliss, A.L. *Metal Ions in Biological Systems*; Marcel Dekker, New York, 1998, Vol. 35, pp 239–327.
6. Neilands, J. B. *J. Biol. Chem.* **1995**, *270*, 26723–26726.
7. Martell, A. E.; Anderson, W.F.; Badman, D.G. (Eds.) *Development of Iron Chelators for Clinical Use*; Elsevier, New York, 1981.
8. McLachlan, D.R.C.; Farnell, B.; Gallin, H.; Karlik, S.; Eichorn, G.; DeBoni, U. *Biological Aspects of Metals and Metal-Related Diseases*; Raven Press, New York, 1983.
9. Yatabe, T.; Kawai, Y.; Oku, T.; Tanaka, H. *Chem. Pharm. Bull.* **1998**, *46*, 966–972.
10. Mock, W. L.; Cheng, H. *Biochemistry* **2000**, *39*, 13945–13952.
11. Brown, D. A.; Roche, A. L. *Inorg. Chem.* **1983**, *22*, 2199–2202.
12. Knežević, A.; Matasić Rossini, M.; Kujundžić, N.; Samaržija, I. *Proceedings of the Second Croatian Congress on Pharmacy*, Cavtat, Croatia, 2001, p. 119.
13. Schwarzenbach, G.; Schwarzenbach, K. *Helv. Chim. Acta* **1963**, *46*, 1390–1395.
14. Kujundžić, N.; Boban, V.; Todorić-Kovačević, V. *Pharmazie* **1992**, *47*, 433–435.
15. Gampp, H.; Maeder, M.; Meyer, C. J.; Zuberbuhler, A. D. *Talanta* **1985**, *32*, 95–101.
16. Gampp, H.; Maeder, M.; Meyer, C. J.; Zuberbuhler, A. D. *Talanta* **1985**, *32*, 257–264.
17. Baes, C. F.; Mesmer, R.E. *The Hydrolysis of Cations*, Wiley, New York, 1976, p. 238, 241.
18. Farkas, E.; Brown, D. A.; Cittaro, R.; Glass, W. K. *J. Chem. Soc. Dalton Trans*. **1993**, 2803–2807.
19. Kurzak, B.; Jezierska, J. *Inorg. Chim. Acta* **1988**, *153*, 193–197.
20. Leporati, E. *J. Chem. Soc. Dalton Trans*. **1986**, 2587–2592.
21. Pećar, M.; Kujundžić, N.; Pazman, J. *J. Pharm. Sci.* **1977**, *66*, 330–333.
22. Pećar, M.; Kujundžić, N.; Mlinarević, B.; Čerina, D.; Horvat, B.; Verić, M. *J. Pharm. Sci.* **1975**, *64*, 970–973.
23. West, T. S. *Complexometry with EDTA and related reagents*; BDH Chemicals Ltd, London, 1969, p. 180, 198.
24. Gans, P.; Sabatini, A.; Vacca, A. *J. Chem. Soc. Dalton Trans*. **1985**, 1195–1200.

Povzetek

S kombinirano pH-spektrometrično titracijo smo študirali komplekse kobalta(II), niklja(II) in bakra(II) s 2-pirolidon-5-hidroksamsko kislino v vodnem mediju s ionsko jakostjo 0,2 M. Kobalt(II) in nikelj(II) tvorita $[ML]^+$, $[ML_2]$ in $[ML_2H_{-1}]^-$ komplekse, medtem ko so prisotne v primeru bakra(II) enojedrne $[CuL]^+$ in dvojedrne $[CuL_2H_{-1}]^-$ zvrsti. Določili smo konstante stabilnosti kompleksov v vodnih raztopinah in opisali mogoče načine vezave. ^{1}H NMR, IR in EPR meritve so dale dodatne informacije o vrsti vezi v dobljenih kompleksih.