

## Copper(II)-aminohydroxamate ternary complexes evidenced by mass spectrometry

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Dedicated to Prof. Harry Lönnberg on the occasion of his 60<sup>th</sup> birthday

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### Abstract

The formation of both binary and ternary 12-metallacrown-4 complexes of  $\alpha$ -aminohydroxamic acids with Cu(II) has been investigated by means of electrospray mass spectrometry, potentiometry and UV-Vis spectroscopy. The formation of a ternary complex composed of phenylalanine and glycine hydroxamic acids was found to be particularly favorable. Among metal used to obtain metallacrowns only Cu(II) was found to be appropriate.

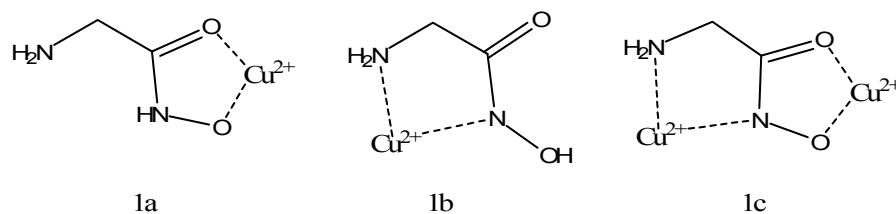
**Keywords:** Metallacrowns, copper(II), hydroxamic acids, ternary complexes, mass spectrometry

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### Introduction

The complexing capacity of hydroxamic acids is of great biological importance, particularly as inhibitors of metalloproteinases<sup>1,2</sup> and as iron chelators.<sup>3,4</sup>

There are several ways of the Cu(II) chelation by  $\alpha$ -aminohydroxamic acids: by the substitution of the proton from the NH-OH group and making an (*O,O*) monohydroxamate chelating ring (Figure 1a), through the deprotonated hydroxamic nitrogen and amino nitrogen (*N,N*) (Figure 1b), or as (*N,N*) – (*O,O*) bridging bis-chelating mode (Figure 1c) with formation of metallacrowns (MC).<sup>5</sup>

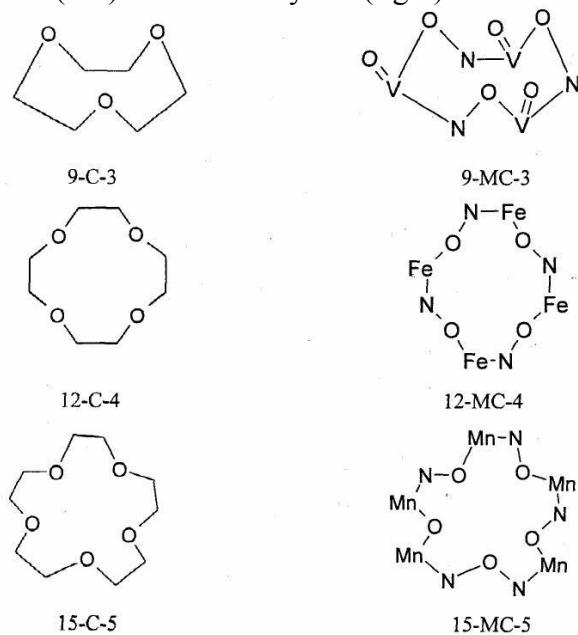


**Figure 1.** Chelating modes of Cu(II) by  $\alpha$ -aminohydroxamic acids

Metallacrowns are a class of metallamacrocycles.<sup>6</sup> The molecular structure of metallamacrocycles (metallacryptates, molecular squares, metallacalixarenes, metallahelicates, and metallacrowns<sup>7,8,9,10,11</sup> resembles that of related organic compounds (cryptates, cyclophanes, calixarenes, helicates, and crown ethers, respectively).

Metallamacrocycles are obtained by replacing carbon atoms of a crown ether by a metal and a heteroatom, bound in the metal's first coordination sphere. A 12-MC-4 can be constructed, by replacing two methylene groups of each macrocycle arm with -M-N- coordination units; in other words, the analogy between the structures of 12-metallacrown-4 and 12-crown-4 is such that both molecules consist of 12-membered macrocycle which offers four oxygen atoms as donors to an ion which can bind in the center of macrocyclic cavity.<sup>12</sup> The sizes of such cavities are similar to each other, although the C-C and C-O and also the M-O and M-N bond lengths are different. It is also worthy to notice that metallacrowns are much more effective in binding a transition metal ion when compared to their organic equivalents, because their oxime oxygen atoms are better donors than the neutral ether oxygen present in the crowns.

In Figure 2, crown ethers (left) and metallacycles (right) are shown for comparison.



**Figure 2.** Schematic analogy between ethers (left) and metallacycles (right)

Metallacrowns can selectively encapsulate metals and anions in their cavities and this particular ability makes them excellent molecular recognition agents.

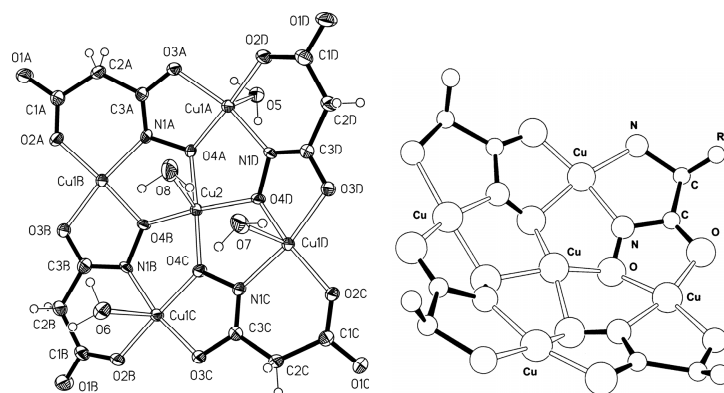
12-MC-4 complexes usually contain Cu(II) in the core of the four-oxygen centre,<sup>13,14</sup> while 15-MC-5 complexes prefer to encapsulate a bigger ion in the five-oxygen cavity (uranyl(II),<sup>15</sup> lanthanide(III)<sup>16</sup> or calcium(II)). The latter are therefore good candidates for applications in the fields of identification and removal of chemical deposits of the cold war or as MRI contrasting agents.

The main advantage of using metallacrowns instead of their organic analogues is the implementation of new geometries, not being restricted to the tetrahedral or trigonal geometry imposed by the carbon atom. Moreover, metallacrowns are formed spontaneously in the solution, with a reasonably high yield and such synthesis doesn't need extreme conditions.

The use of electrospray mass spectrometry (ESI-MS) to characterize the stoichiometry of metal complexes in solution is well documented.<sup>17,18,19</sup> This technique was found to be also very useful in the studies of metallacrowns formed by aminohydroxamic acids and Cu(II). The ESI-MS results confirmed the presence of a pentameric metallacrown species  $[\text{Cu}_5\text{L}_4\text{H}_4]^{2+}$  rather than binuclear one  $[\text{Cu}_2\text{L}_2\text{H}_1]^-$  in the most studied systems.<sup>20,21,22,23,24</sup>

## Results and Discussion

The formation of the pentameric 12-MC-4 Cu(II) complexes of  $\alpha$ - and  $\beta$ -aminohydroxamic acids is well documented in the earlier studies.<sup>20,21,22,25,26,27</sup> Moreover, the solution and X-ray studies on the Cu(II) complexes of malonomonohydroxamic acid have demonstrated the first example of a Cu(II) 12-MC-4 hydroxamic metallacrown compound formed by the hydroxamic ligand having an additional carboxyl donor function placed in the  $\beta$ - position with respect to the hydroxamic group.<sup>28</sup> Examples of the structures corresponding to pentanuclear Cu(II) complexes of  $\alpha$ -aminohydroxamic and malonomonohydroxamic acids are presented in Figure 3.



**Figure 3.** From the left: the planar structure of the 12-MC-4 complex of Cu(II) and malonomonohydroxamate; a scheme of the cup-shaped 12-MC-4 based on Cu(II) and  $\alpha$ -aminohydroxamic acids.<sup>20</sup>

Since only binary complexes have been studied, there is not much evidence on the hydroxamic acid mixed pentanuclear metallaorganic complexes. Therefore, looking for the formation of the ternary species, we have reinvestigated Cu(II) :  $\alpha$ -aminohydroxamic acids (namely Cu(II) : glycinehydroxamate : phenylalanine-hydroxamate) system. In order to follow the stoichiometry of the complexes and to examine their stabilities we have used ESI-MS together with potentiometry and UV-Vis spectroscopy.

In order to study the formation of the ternary metallacrowns based on Cu(II) mixed glycine (Glyha) and phenylalanine (Pheha) hydroxamic acids, first we have performed solution studies of the binary Cu(II):Glyha and Cu(II):Pheha systems under our experimental conditions.

ESI-MS spectra of Cu(II) complexes with phenylalanine hydroxamic acid have shown the formation of three species (Table 1). The major signal, at  $m/z$  of 513.477, came from the pentameric  $[\text{Cu}_5\text{Pheha}_4\text{H}_4]^{2+}$  complex. Another signal assigned to metallacrown was seen at 1125.910 and it derived from its adduct with perchlorate ion ( $[\text{Cu}_5\text{Pheha}_4\text{H}_4]\text{ClO}_4^+$ ). Because the measurements were performed at pH 5, a peak of  $[\text{CuPheha}_2]\text{H}^+$  complex was also observed at 422.100. The calculated pseudomolecular ions (Table 1) are in perfect agreement with the simulated monoisotopic masses. In the negative ionization mode, no significant bands were observed. These results are in line with the literature data.

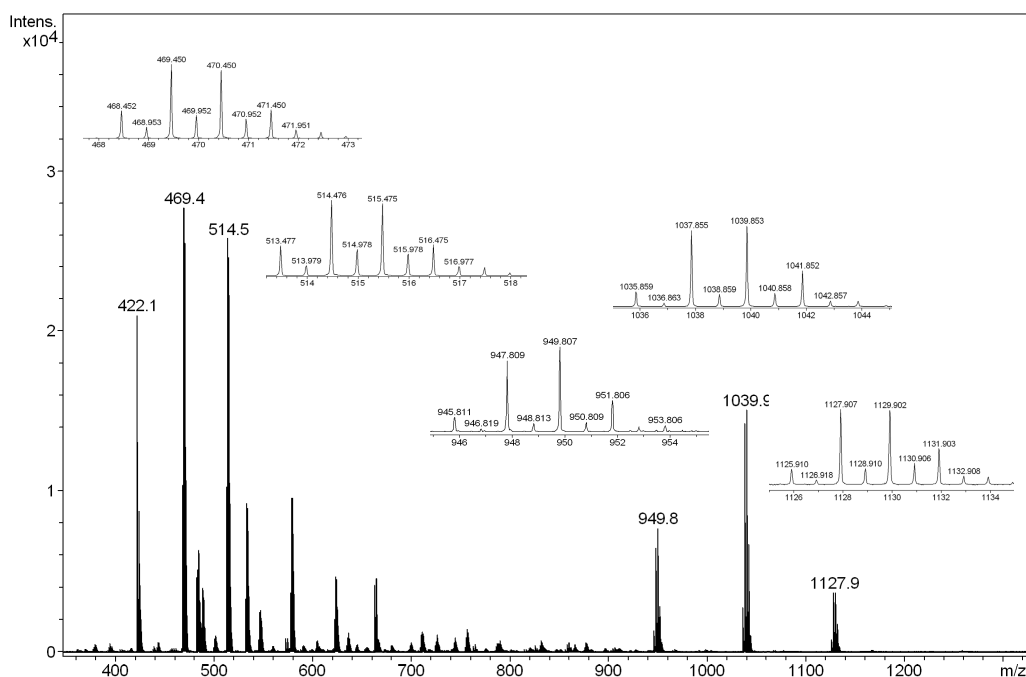
Under our experimental conditions for glycinehydroxamate copper(II) system only pentameric complex  $[\text{Cu}_5\text{Glyha}_4\text{H}_4]\text{ClO}_4^+$  was observed ( $m/z = 767.701$ , Table 1). Probably due to a weak ESMS response, the bis-complex  $[\text{CuGlyha}_2]\text{H}^+$  could not be seen.

**Table 1.** Detected major ESI-MS ions for Cu(II)-L (L = glycinehydroxamate and phenylalaninehydroxamate) complexes and adducts\*

Aminohydroxamic acid	Pseudomolecular Ion	$m/z$ (found / calculated)	Relative intensity
glycinehydroxamate	$[\text{Cu}_5\text{Glyha}_4\text{H}_4]\text{ClO}_4^+$	767.701/767.702	-
phenylalaninehydroxamate	$[\text{CuPheha}_2]\text{H}^+$	422.100 / 422.101	39%
	$[\text{Cu}_5\text{Pheha}_4\text{H}_4]^{2+}$	513.477 / 513.472	100%
	$[\text{Cu}_5\text{Pheha}_4\text{H}_4]\text{ClO}_4^+$	1125.910 / 1125.893	21%

\*  $m/z$  values of monoisotopic peaks are presented. Solvent: MeOH/H<sub>2</sub>O 1/1 v/v.

For the Cu(II):Pheha:Glyha system of 1:1:1 metal-to-ligands molar ratio, the ESI-MS spectrum clearly indicated the formation of the ternary complexes. The original spectrum is shown on Figure 4 together with the zoomed monoisotopic peaks and the pseudomolecular ions of the various observed species are collected in Table 2.



**Figure 4.** ESI-MS spectrum obtained for Cu(II):Pheha:Glyha system with molar ratio of 1:1:1 ([Cu(II)] =  $10^{-4}$ M). Solvent: MeOH/H<sub>2</sub>O 1/1 v/v.

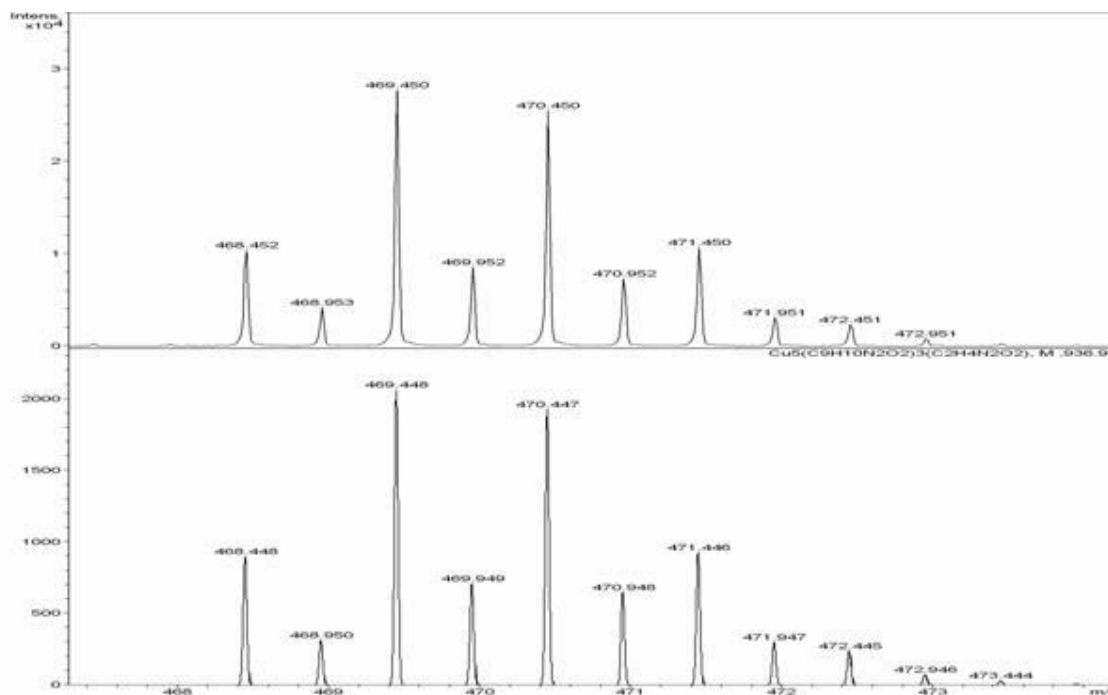
[Cu(Pheha)<sub>2</sub>]<sup>+</sup> *m/z* 422.100, [Cu<sub>5</sub>GlyhaPheha<sub>3</sub>H<sub>4</sub>]<sup>2+</sup> *m/z* 468.452, [Cu<sub>5</sub>(Pheha)<sub>4</sub>H<sub>4</sub>]<sup>2+</sup> *m/z* 513.477, [Cu<sub>5</sub>Glyha<sub>2</sub>Pheha<sub>2</sub>ClO<sub>4</sub>H<sub>4</sub>]<sup>+</sup> *m/z* 945.811, [Cu<sub>5</sub>GlyhaPheha<sub>3</sub>ClO<sub>4</sub>H<sub>4</sub>]<sup>+</sup> *m/z* 1035.859, [Cu<sub>5</sub>(Pheha)<sub>4</sub>ClO<sub>4</sub>H<sub>4</sub>]<sup>+</sup> *m/z* 1125.910.

**Table 2.** Detected major ESI-MS ions for ternary Cu(II):Pheha:Glyha system (1:1:1)\*

Pseudomolecular Ion	<i>m/z</i> (found / calculated)	Relative intensity
[CuPheha <sub>2</sub> ] <sup>+</sup>	422.100 / 422.101	77%
[Cu <sub>5</sub> Pheha <sub>4</sub> H <sub>4</sub> ] <sup>2+</sup>	513.477 / 513.472	93%
[Cu <sub>5</sub> Pheha <sub>4</sub> H <sub>4</sub> ] <sup>+</sup> ClO <sub>4</sub> <sup>-</sup>	1125.910 / 1125.893	14%
[Cu <sub>5</sub> GlyhaPheha <sub>3</sub> H <sub>4</sub> ] <sup>2+</sup>	468.452 / 468.448	100%
[Cu <sub>5</sub> GlyhaPheha <sub>3</sub> H <sub>4</sub> ] <sup>+</sup> ClO <sub>4</sub> <sup>-</sup>	1035.859 / 1035.846	53%
[Cu <sub>5</sub> Glyha <sub>2</sub> Pheha <sub>2</sub> H <sub>4</sub> ] <sup>+</sup> ClO <sub>4</sub> <sup>-</sup>	945.811 / 945.799	27%

\* *m/z* values of monoisotopic peaks are presented. Solvent: MeOH/H<sub>2</sub>O 1/1 v/v.

On the spectrum of the ternary system (Figure 4), the most intense signal corresponds to a ternary copper complex containing one glycinehydroxamic acid and three phenylalanine hydroxamic acid molecules: [Cu<sub>5</sub>GlyhaPheha<sub>3</sub>H<sub>4</sub>]<sup>2+</sup>, *m/z* of 468.452. The experimental and simulated ESI-MS spectra are in perfect agreement and are shown in Figure 5.



**Figure 5.** Top: The ESI-MS spectrum of peaks corresponding to  $[\text{Cu}_5\text{GlyhaPheha}_3\text{H}_4]^{2+}$  Bottom: The simulated isotopic pattern of the complex.

The peak corresponding to the pentameric binary  $[\text{Cu}_5\text{Pheha}_4\text{H}_4]^{2+}$  complex (513.477) was slightly less intense. The  $\text{ClO}_4^-$  ionized adducts of both, binary  $[\text{Cu}_5\text{Pheha}_4\text{H}_4]\text{ClO}_4^+$  (1125.910) and ternary  $[\text{Cu}_5\text{GlyhaPheha}_3\text{H}_4]\text{ClO}_4^+$  species were significantly less intense. Another ternary complex with metal-to-ligands ratio of 5:2:2 was visible only as perchlorate adduct  $[\text{Cu}_5\text{Glyha}_2\text{Pheha}_2\text{H}_4]\text{ClO}_4^+$  at  $m/z$  of 945.811. Although statistically this should be the most abundant form in the solution of metallacrowns, the intensity of its signal is much lower than the intensity of signals derived from the Cu(II):Glyha:Pheha 5:1:3 species. However, one has to remember that the relative intensities of the ions in an ESI-MS spectrum do not necessarily reflect the relative concentrations of the ions in solution.<sup>29</sup>

The monomeric  $[\text{CuPheha}_2]\text{H}^+$  species give a signal at 422.100. No evidence for the formation of the analogues species with Glyha was seen, neither for the mixed bis-complex  $\text{Cu}[\text{GlyhaPheha}]\text{H}^+$ .

In order to check the stability of the studied metallacrown complexes we have carried out MS/MS experiments, which revealed that the decomposition of the noncovalent complexes took place at relatively high CID energies (over 20eV).<sup>30</sup> Moreover, experiments performed eight weeks after the samples have been prepared give the same results as on the freshly prepared ones.

The outstanding intensity of the  $[\text{Cu}_5\text{GlyhaPheha}_3\text{H}_4]^{2+}$  complex has been further studied by potentiometry and UV-Vis spectroscopy. Mass spectrometric and potentiometric results are complementary to each other and give an idea about the true nuclearity of the complexes and

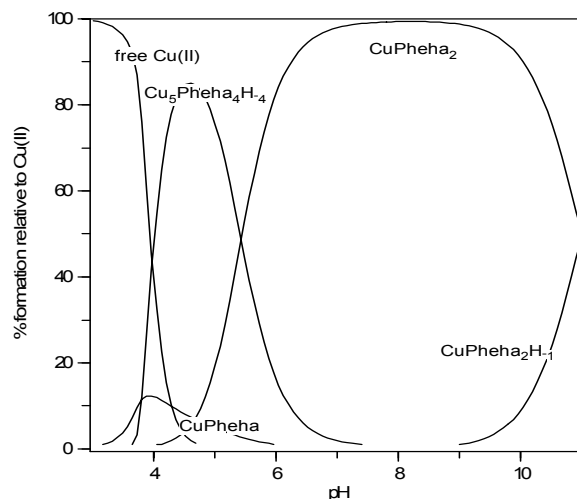
about the dominant species in the solution. Before the solution studies of the ternary system were performed, first the binary systems were reexamined under our experimental conditions. Protonation constants of the two ligands and complex formation constants obtained for the three systems (Cu(II)-Glyha, Cu(II)-Pheha and Cu(II)-Glyha-Pheha) are reported in Table 3 together with the literature data. For the binary systems, a four species model has been calculated ( $[LH]$ ,  $[LH_2]^+$ ,  $[CuL]$ ,  $[Cu_5L_4H_{-4}]^{2+}$ ,  $[CuL_2]$ ,  $[CuL_2H_{-1}]^-$ ) what is in accordance with both, the mass spectrometric results reported herein and the literature data<sup>22,27</sup> For both investigated ligands the obtained experimental values are in good agreement with the literature data.<sup>22,27</sup> Low solubility of the phenylalaninehydroxamic acid might be the reason why these complexes have not been frequently studied in the aqueous solution. As expected, the values obtained in aqueous solution are slightly lower than those reported for methanol (Table 3). The increased stability of the Cu(II) complexes in methanol with respect to the aqueous solution can be attributed both, to the increased basicity of the hydroxamate group and to more favorable solvation effects.

**Table 3.** Logarithms of protonation and Cu(II) complex formation constants for glycinehydroxamic acid, phenylalaninehydroxamic acid and their ternary system

Species	log $\beta$		
	This work	Literature data	
[GlyhaH]	9.71(4)	9.18 <sup>a</sup>	
[GlyhaH <sub>2</sub> ] <sup>+</sup>	17.38(5)	16.60 <sup>a</sup>	
[CuGlyha] <sup>+</sup>	11.33(4)	10.51 <sup>a</sup>	
[Cu <sub>5</sub> Glyha <sub>4</sub> H <sub>-4</sub> ] <sup>2+</sup>	42.75(4)	39.96 <sup>a</sup>	
[CuGlyha <sub>2</sub> ]	21.42(2)	20.34 <sup>a</sup>	
[CuGlyha <sub>2</sub> H <sub>-1</sub> ] <sup>-</sup>	11.05(3)	11.07 <sup>a</sup>	
[PhehaH]	9.83(1)	9.01 <sup>b</sup>	10.75 <sup>c</sup>
[PhehaH <sub>2</sub> ] <sup>+</sup>	17.01(2)	15.9 <sup>b</sup>	17.80 <sup>c</sup>
[CuPheha] <sup>+</sup>	11.34(5)		13.45 <sup>c</sup>
[Cu <sub>5</sub> Pheha <sub>4</sub> H <sub>-4</sub> ] <sup>2+</sup>	44.42(3)		51.58 <sup>c</sup>
[CuPheha <sub>2</sub> ]	21.97(1)	19.78 <sup>b</sup>	23.11 <sup>c</sup>
[CuPheha <sub>2</sub> H <sub>-1</sub> ] <sup>-</sup>	10.96(3)		10.98 <sup>c</sup>
[Cu <sub>5</sub> GlyhaPheha <sub>3</sub> H <sub>-4</sub> ] <sup>2+</sup>	44.89(7) <sup>d</sup>		

I = 0.1 M (KNO<sub>3</sub>), T = (25.0 ± 0.2)°C. The reported errors on log $\beta$  are given as 1 $\sigma$ ,<sup>a</sup> ref.<sup>27</sup>,<sup>b</sup> ref., in methanol, ref.<sup>26</sup>,<sup>d</sup> for the ternary system log $\beta$  for binary Cu(II)-Glyha and Cu(II)-Pheha complexes were kept constant.

As an example we present a distribution diagram for Cu(II)-phenylalaninehydroxamic acid system (Figure 6). For the simplicity reasons the charges of the complexes were omitted.

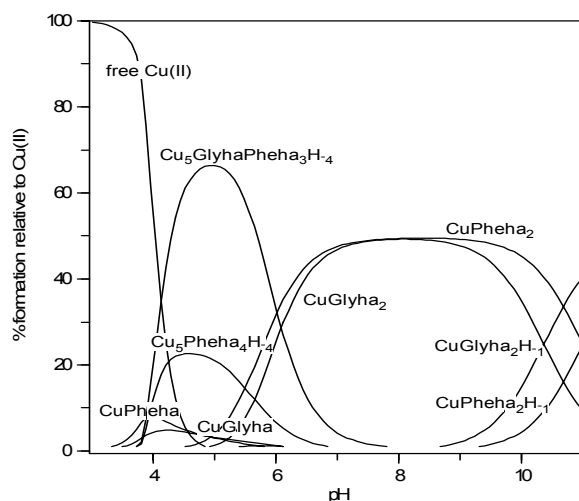


**Figure 6.** Distribution diagram of the Cu(II):Pheha system (Cu(II):Pheha=1:3,  $[Cu(II)] = 1.5 \times 10^{-3}$  M).

The first complex formed at relatively low pH is  $[CuL]^+$ , with a formation constant of about 11.3 and a maximum abundance at pH 4. At pH 5, the major species present in the solution is the pentameric  $[Cu_5L_4H_4]^{2+}$  metallacrown. The main difference in the formation constants for Glyha and Pheha is the  $\log\beta$  of the metallacomplexes, with the constant being 1.67 units higher for  $[Cu_5Pheha_4H_4]^{2+}$  when compared to  $[Cu_5Glyha_4H_4]^{2+}$ . In the pH range 6.5-9.5,  $CuL_2$  becomes a dominant species, with a  $\log\beta$  of about 21.9. Above pH 11  $[CuL_2H_1]$  is the major complex.

For the ternary systems the potentiometric titrations were performed for solution containing Cu(II), Glyha and Pheha with metal-to-ligand molar ratio of 2 : 3 : 3. As far as the ternary complexes are concerned, mass spectrometry data suggested mainly the formation of a ternary  $[Cu_5GlyhaPheha_3H_4]^{2+}$  species. However, the signal with lower intensity was seen also for  $[Cu_5Glyha_2Pheha_2H_4]ClO_4^+$  complex (Figure 4). In the calculations based on the potentiometric data the assumption of the ternary  $[Cu_5Glyha_2Pheha_2H_4]^{2+}$  species has not lead to a satisfactory fitting of the titration curves. The fitting was much improved when the  $[Cu_5GlyhaPheha_3H_4]^{2+}$  complex was introduced into the model and a stability constant of  $\log K = 44.89(7)$  was calculated. This proves that in this case the low intensity signal in the mass spectrum clearly indicates the minor concentration of the particular species in the solution. That is why the  $[Cu_5Glyha_2Pheha_2H_4]^{2+}$  species have not been taken into account in the distribution diagram of the Cu(II) – Glyha – Pheha system presented on Figure 7.





**Figure 7.** Distribution diagram of the Cu(II):Glyha:Pheha ternary system (Cu(II):Glyha:Pheha = 2:3:3,  $[\text{Cu(II)}] = 1.5 \times 10^{-3} \text{M}$ )

Due to the fact that the  $[\text{CuL}]^+$ ,  $[\text{CuL}_2]$  and  $[\text{CuL}_2\text{H}_{-1}]^-$  species for both Glyha and Pheha have similar formation constants, their species distributions are quite similar to each other. The difference in  $\log\beta$  value (1.67 units) was found between  $[\text{Cu}_5\text{Pheha}_4\text{H}_{-4}]^{2+}$  ( $\log\beta = 44.42$ ) and  $[\text{Cu}_5\text{Glyha}_4\text{H}_{-4}]^{2+}$  ( $\log\beta = 42.75$ ). That is the reason for which the abundance of the particular metallacrowns differs in the mixed ligand solution. The data obtained in potentiometric measurements are in perfect agreement with the MS results, the ternary  $[\text{Cu}_5\text{GlyhaPheha}_3\text{H}_{-4}]^{2+}$  complex is the major species found in solution ( $\log\beta = 44.89$ );  $[\text{Cu}_5\text{Pheha}_4\text{H}_{-4}]^{2+}$  seems to be less abundant, while the  $[\text{Cu}_5\text{Glyha}_4\text{H}_{-4}]^{2+}$  is a minor species.

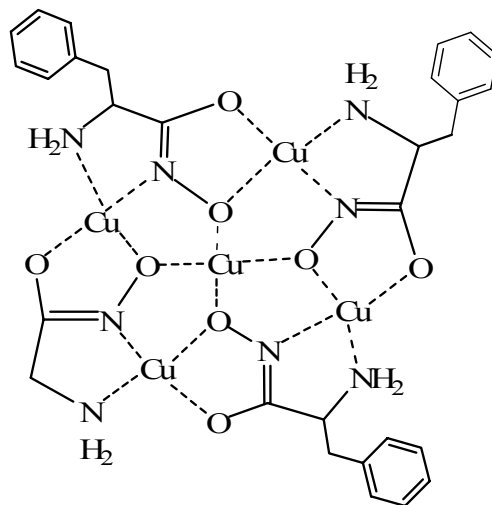
Solution spectra for Cu(II)-Glyha and Cu(II)-Pheha were recorded at appropriate pH for the formation of the binary (Cu(II):L = 1:3) and ternary (Cu(II):Glyha:Pheha = 2:3:3) systems. The visible spectral features of  $[\text{CuL}]^+$ ,  $[\text{Cu}_5\text{L}_4\text{H}_{-4}]^{2+}$ ,  $[\text{CuL}_2]$  and  $[\text{CuL}_2\text{H}_{-1}]^-$  for both of the ligands are in agreement with those reported for other  $\alpha$ -aminohydroxamates in aqueous solution.<sup>26,20</sup> At pH above 2, the spectroscopic parameters reveal the presence of d-d transitions (at about 800nm), characteristic for copper aqua ions. At pH about 4, a low absorption d-d band is observed at a wavelength of about 700nm, which corresponds to the expected [N,N]-bidentate  $[\text{CuL}]^+$  species. This binding mode changes at pH 5, when the [N,N]-[O,O]-chelating  $[\text{Cu}_5\text{L}_4\text{H}_{-4}]^{2+}$  complex begins to dominate in the solution, giving strong absorption spectra at a wavelength of about 648nm. As the pH further increases (above 6), the  $[\text{CuL}_2]$  form begins to appear giving a broad spectrum with a maximum at 529nm ( $\epsilon = 74$ ). A slight hypsochromic shift (28nm) is observed at pH 10, when going on from the  $[\text{CuL}_2]$  to the  $[\text{CuL}_2\text{H}_{-1}]^-$  form. This may be the result from the deprotonation of the -NOH group. UV-Vis characteristics of the complexes are collected in Table 4.

**Table 4.** UV-Vis spectroscopic parameters for Cu(II): Glyha: Pheha (2:3:3) system

Ligand	Species	pH	$\lambda_{\max}$ (nm)	$\epsilon$ (mol <sup>-1</sup> dm <sup>3</sup> cm <sup>-1</sup> )
Glyha	[CuL] <sup>+</sup>	3.91	694.5	42.38
	[Cu <sub>5</sub> L <sub>4</sub> H <sub>4</sub> ] <sup>2+</sup>	4.94	649.0	416.00
	CuL <sub>2</sub>	8.17	530.0	84.18
	[CuL <sub>2</sub> H <sub>1</sub> ] <sup>-</sup>	11.07	498.0	98.65
Pheha	[CuL] <sup>+</sup>	4.09	718.0	28.42
	[Cu <sub>5</sub> L <sub>4</sub> H <sub>4</sub> ] <sup>2+</sup>	5.04	646.0	380.80
	CuL <sub>2</sub>	7.93	524.0	72.86
	[CuL <sub>2</sub> H <sub>1</sub> ] <sup>-</sup>	10.11	509.5	80.05
Glyha + Pheha	[CuL] <sup>+</sup>	3.95	712.0	33.79
	[Cu <sub>5</sub> L <sub>4</sub> H <sub>4</sub> ] <sup>2+</sup>	4.90	648.0	403.36
	CuL <sub>2</sub>	8.11	529.0	73.63
	[CuL <sub>2</sub> H <sub>1</sub> ] <sup>-</sup>	11.12	501.5	84.50

$I = 0.1$  M (KNO<sub>3</sub>),  $T = (25.0 \pm 0.2)^\circ\text{C}$ . The errors are:  $\lambda_{\max} \pm 2\text{nm}$  and  $\epsilon \pm 10\%$ .

Potentiometric data permitted to determine the stoichiometry and the stability constants of the complexes formed in solution. Mass spectrometry confirmed the exact molecular mass of the complexes and allowed to suggest their structure (Figure 8).

**Figure 8.** The ternary [Cu<sub>5</sub>GlyhaPheha<sub>3</sub>H<sub>4</sub>]<sup>2+</sup> complex

High affinity of hydroxamic acids towards copper and their excellent ability to form 12-metallacrown-4 complexes has already been shown. We have also reinvestigated other transition metals (Fe(III), Zn(II), Ni(II)) to test their potential ability to form 12-MC-4 complexes with hydroxamic acids. The potentiometric and ESI-MS experimental results<sup>31</sup> confirm that copper

seems to be the only metal capable of forming stable 12-metallacrown-4 complexes with  $\alpha$ -aminohydroxamic acids studied, what is in perfect agreement with the literature data.<sup>32,33,34</sup>

## Conclusions

Phenylalanine and glycine hydroxamic acids form with Cu(II) ions metallacrown species both for binary and ternary systems. The most stable macrocyclic species in the case of ternary complexes seems to be  $[\text{Cu}_5\text{GlyhaPheha}_3\text{H}_4]^{2+}$  species. Other hydroxamates like  $\beta$ -alaninehydroxamic or malonomonohydroxamic acids are also able to form very stable ternary species of composition  $[\text{Cu}_5\beta\text{-AlahaMacz}_3\text{H}_4]^-$  ( $m/z = 766.69$ ) and detailed investigations are in progress.

## Experimental Section

**General Procedures.** Two of the ligands- glycine hydroxamic acid (Glyha) and phenylalanine hydroxamic acid (Pheha), the  $\text{Cu}(\text{ClO}_4)_2$  salt and all solvents were of analytical grade and were used without further purification. Both hydroxamic acids were purchased from Sigma Aldrich.

Mass spectra were obtained on a Bruker Microtof-Q spectrometer (Bruker Daltonik, Bremen, Germany), equipped with Apollo II electrospray ionization source with ion funnel. The mass spectrometer was operated in the positive ion mode. The instrumental parameters were as follows: scan range  $m/z$  300–1600, end plate offset -500 V, dry gas – nitrogen (4 L/min), temperature 200 °C, ion energy 5 eV. Capillary voltage was optimized to the highest S/N ratio and it was 4500 V. The small changes of voltage ( $\pm 500\text{V}$ ) did not significantly affect the optimized spectra. The sample ( $\text{Cu}(\text{ClO}_4)_2$ , Glyha and/or Pheha with 1:1:1 stoichiometry,  $10^{-4}\text{M}$ ) was prepared in 1:1 MeOH- $\text{H}_2\text{O}$  mixture. Variation of the solvent composition down to 5% of MeOH did not change the species composition. The sample was infused at a flow rate of 3  $\mu\text{L}/\text{min}$ . The instrument was calibrated externally with the Tunemix™ mixture (Bruker Daltonik, Germany) in quadratic regression mode. Data were processed by using the Bruker Compass DataAnalysis 4.0 program.

The potentiometric titrations were performed using a Dosimat 665 Metrohm titrator connected to a Metrohm 691 pH-meter and a Metrohm LL Unitrode glass electrode. The thermostabilized glass-cell was equipped with a magnetic stirring system, a microburet delivery tube and an inlet-outlet tube for Argon. The solutions at 0.1M ionic strength in  $\text{KNO}_3$  were titrated at 25.0 °C with 0.1M carbonate-free KOH. The electrodes were daily calibrated for hydrogen ion concentration by titrating HCl with KOH in the same experimental conditions as above. The daily calibration results were processed with Gran method.<sup>35</sup> To evaluate complex formation constants of Cu(II) with Glyha or/and Pheha the solutions of metal-to-ligand(s) molar ratios of 1:3 and 1:1:1, 2:3:3 and 1:1:3 at constant Cu(II) concentrations of  $1.5 \times 10^{-3}\text{M}$  were

examined. All the pH-potentiometric titrations were performed over the pH range of 2–11. Acid and complex formation constants were calculated using Hyperquad 2006 computer programme<sup>36</sup> which minimizes the sum of the weighted squared residuals between the observed and the calculated e.m.f. values. Distribution diagrams for the various systems were calculated and plotted by the programme HYSS<sup>37</sup>.

UV-Vis absorption spectra were recorded with a Cary 300 Bio spectrometer, equipped with quartz cells of 1 cm path length, between 400 and 800 nm. The Cu(II) to ligand(s) molar ratio was 1:3 for the binary and 2:3:3 (Cu(II):Glyha:Pheha) for the ternary systems, with the concentration of the ligand being  $1 \times 10^{-3}$  M. A series of measurements were performed in the pH range of 2-11.

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