

MONIKA TUREK^a**XIANA LAVIANA SENAR^b**^a**Institute of General Food Chemistry, Technical University of Lodz**^b**Universiad Autonomia de Barcelona, Biociencias**

POTENTIOMETRIC AND SPECTROSCOPIC STUDIES ON DI-, TRI- AND TETRAGLYCINE WITH COPPER (II) IONS SYSTEMS

Review: Elżbieta Łodyga-Chruścińska, Ph. D., D. Sc.

This article is a review of literature on copper (II) ions with di-, tri- and tetraglycine complexes, with special reference to kinds of their complexing forms. Potentiometric and spectroscopic studies of the complexes mentioned above have also been performed. Additionally, a dependence of formation of individual complexes on pH has been demonstrated.

1. Introduction

Copper from *cuprum* (Cu) is the 29th element of the Periodic Table with electronic configuration $3d^{10}4s^1$. It is a transient metal with three oxidation states: Cu^0 , Cu^{1+} and Cu^{2+} . Cu^0 is difficult to oxidize because of its high electrode potential, therefore copper is often found in nature in the metallic state. The most important oxidation state in natural, aqueous environment is copper(II) or cupric state [1].

Copper is an essential trace element present in the parts per million concentration in the organism. The chemical properties of copper are the stability of its redox-interchangeable prevalent ionic forms, its ability to bind oxygen and its affinity for functional groups that occur in proteins. Copper exists in a complex form with proteins, peptides, and amino acids and probably other organic substances in the organism [1, 2].

2. Copper in biological system

Copper proteins are widely distributed in living organisms. They are found in electron transfer proteins (azurin, plastocyanin, laccase), in enzymes that bind oxygen, such as oxidases (ferroxidase, tyrosinase, ascorbate oxidase), and in oxygen transport proteins (hemocyanin) [1].

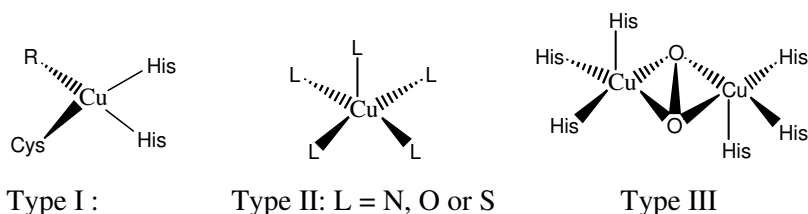
There have been numerous experiments referring to copper proteins with X-ray that provide detailed information on the coordination environment of active centers. Copper is located in the matrix surrounded by the polypeptide chain and bound with the functional groups from the chain, in most cases histidine imidazoles. Proteins also provide these bonds (N-terminal nitrogen atom) [3].

The copper sites in the redox proteins have historically been divided into three classes based on their spectroscopic features, which reflect the geometric and electronic structure of the active site: type I (T1) or blue copper, type II (T2) or normal copper, and type III (T3) or coupled binuclear copper centres. Over the last 20 years, these sites have expanded to include multicopper oxydases (comprising type II and III centres), Cu_A and Cu_B -heme_A centre of cytochrome *c* oxidase and Cu_Z centre of nitrous oxide reductase [4] (1).

Type I (T1): Called blue copper proteins due their intense absorbance (ϵ between 3500 and 6000 $\text{M}^{-1} \text{cm}^{-1}$) at ~600 nm. This band is assigned as a cysteine sulphur to copper ligand-to-metal charge transfer (LMCT) transition. Type I centers also exhibit a very small Cu parallel hyperfine splitting in their EPR spectra due to the high covalency at the copper site. These sites are found in mononuclear copper proteins involved in intermolecular electron transfer pathways and the multicopper enzymes, laccase, ceruloplasmin, and nitrite reductase, where they function in intramolecular electron [4].

Type II (T2): The copper centres in these proteins are spectroscopically consistent with square planar, pyramidal five-coordinate, or tetragonally distorted octahedral geometries, containing oxygen and/or nitrogen ligation. They have larger EPR signals similar to those of tetragonal Cu(II) complexes and are often characterized by relatively weak absorption spectra, associated with ligand field transitions. There is no intense blue colour. This group includes the copper/zinc superoxide dismutase dopamine b-monooxygenase, galactose oxidase and the various copper-containing amine oxidases [4].

Type III (T3): type III centre contains two ligand-bridge copper ions called a binuclear site, EPR silent in the oxidized Cu(II)-Cu(II) state. They participate in dioxygen transport, dioxygen activation and oxygenation reactions [4].



R = S_{Met} (azurin plastocyanin, laccase), O_{Glu} (phytyocyanins), H₂O (ceruloplasmin)

Different types of copper centre geometries (I) [5]

Finally, there are further groups, Cu_a with dinuclear disposition and Cu_b with mononuclear disposition that are found in cytochrome c oxidase and the binuclear Cu_z centre of nitrous oxide reductase [4, 5].

Copper is indispensable to our existence since the beginning of the formation of life. Before life emerged on earth around 3.5-3.8 billion years ago Cu(II)/amino acid complexes had played a crucial role in peptide formation thanks to their catalytic effect, the mechanism is a so called “salt-induced peptide formation” (SIPF). SIPF permitted formation of the first oligopeptides and peptides which played a very important role at that time, as they were the first environmental relatively stable catalysts to carry the essential information [6].

The SIPF reaction was discovered in 1989, and there is a strong evidence that SIPF reaction was responsible for the production of the first peptides on primitive earth, where there were all components necessary for that condensation reaction. This reaction took place in the hot salty oceans, lakes, lagunas, and puddles after the first condensation of water on our planet. The system contained a high concentration of sodium chloride, amino acids, copper ions at the temperature around 80°C [6].

The experiments referring to the mechanism of the reaction are based on the formation of a monochlorocuprate complex with two amino acids (or one peptide and one amino acid) to create a new peptide linkage between the reactants. A nucleophilic attack of the amino group of the chelated amino acid at the protonated amino acids carbonyl carbon. A important feature of these reactions is the strong affinity of the biologically relevant α- over β- and γ-amino acids due to their better complex formation ability with catalytic Cu(II) ion [6, 7].

3. Oligopeptides as ligands for copper (II) IONS

Oligopeptides are molecules of low symmetry and with three or more functional groups, which are able to associate with protons. Cu (II) forms very stable complexes with simple oligopeptides. The modes of coordination of copper with simple oligopeptides have been studied in detail [8, 9].

The investigation of metal complexes with oligopeptides can enhance our understanding of the role of metal ion-protein. As it has been said before, various metal ions play an important role in the binding and transport of organic molecules and in the catalysis of different acid-base and redox processes in biological systems. Many proteins and enzymes in living systems have one or more metal ions bound to them which are essential for their activity. The understanding of interactions between amino acids and metals is therefore very important to understand the function of this complex or metalloproteins [8].

The physiological role of polyfunctional biomolecules is strictly structure-dependent. Furthermore, the type and extended metal-ligand interactions are considerably influenced by the protonation stage of the ligand. Changes in the protonation of macromolecular polypeptides or protein may result, for example, in the formation or cleavage of intramolecular H-bonds, which stabilize the secondary and tertiary structure of the molecule. Consequently, the knowledge of proton-binding characteristics is of primary importance for understanding, of metal-ligand interactions [10].

The structure and stability of the complexes are controlled by stereochemistry of metal site, the nature of the ligands attached to the metal and the protein environment, which plays a crucial role in controlling the metal site reactivity. The environment is affected by such factors as temperature, the kind of solvent (water, ethanol, and others), the interaction enthalpies, entropies, and Gibbs energies, etc. The knowledge of computed Gibbs energies ΔG° is important to realize how strong are the complexes. In some cases the protein can force metal ions into unusual geometries; the protein environment may be a determining factor which controls the activity of the increasing number of functionally distinct metalloproteins that have essentially identical metal centres [11, 12].

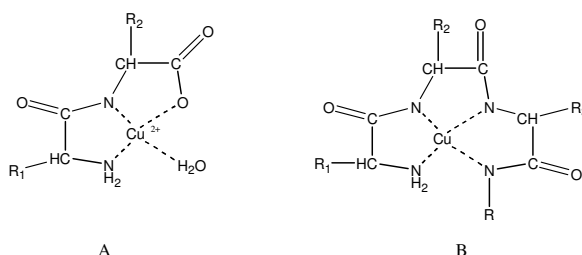
Amino acids can form bis or tris complexes with various transition metal ions, where the coordination takes place *via* α -amino and carboxylate groups so as to form 5-membered chelate rings. The type of coordination is affected by side chains. According to the steric requirements and polar position of the amino and carboxylate groups the complex formation excludes to five-membered chelate rings. At least four groups, all of them capable of metal ion coordination, are present in dipeptide, amino-N, carbonyl-O, amide-N, and carboxylate-O, which is referred to as peptide linkage. The other donor group can be in the side chain of molecule (R), which results in great variety of metal ion-peptide interactions. The basicity of donor groups in peptide molecules is one of the most important parameters influencing the coordination chemistry of oligopeptides [10].

As for the acid-base properties, it is clearly demonstrated that if the side chains do not contain any additional functional groups (like glycine), only two pK values are measured in the pH range 0-14: a pK around 3 attributable to $-\text{COOH}$ group, and another around 8 attributable to $-\text{NH}_3^+$ group [10].

As it has been mentioned, there are at least four donor atoms in a dipeptide and only three functional groups; the reason is that the amide group offers two potential donor atoms, carbonyl-O and amide-N. However, tetrahedral amino nitrogen which

possesses a lone pair of electrons in the amino acid (sp^3), loses its basicity when it reacts to give an amide group (trigonal nitrogen sp^2), because amide groups are planar with a double-bond character of the carbon-nitrogen bond. The coordination of an amide bound takes place in a very basic solution in the absence of a metal ion proton, which is normally possible only after its deprotonation [9, 10].

The terminal amino and carboxylate groups are the most effective binding site for the metal ions in oligopeptides to cause the “neutrality” of the amide group. So, amino and carboxylate groups are independent primary binding groups or ‘anchors’ for metal ion binding, and their interaction will depend on steric requirements and the nature of metal ion. Moreover, the amino-N and carbonyl-O donor atoms are in the position to have the capacity to form five-membered chelates enhancing the thermodynamic stability of peptide complexes and avoiding the precipitation of metal hydroxide [11, 13, 14].



Five-membered chelate rings: (A) Dipeptide, (B) Tetrapeptide (2) [15]

That formation also favours the deprotonation and coordination of amide group via the nitrogen atom $-N$ which is in a chelatable position towards $Cu(II)$. A significantly stronger binding is achieved when the amide nitrogen is involved. The deprotonation and metal ion coordination of the amide function is further supported by the formation of another five-membered chelate ring including the terminal carboxylate group in the case of dipeptides (**2A**) [15, 16].

In the case of tripeptides or the longer counterparts, the second and third amide functions can also be deprotonated saturating the equatorial coordination sites of the metal ions (**2B**). Copper (II) generally forms the common tetragonally distorted octahedral complexes with any simple oligopeptides. The coordination of the kind of metal ions such as Cu^{2+} starts at the N-terminal amino nitrogen. The adjacent carbonyl oxygen is the second donor to complete the chelate ring. By raising the pH, the metal ions are able to deprotonate successive peptide nitrogens forming $M-N$ bonds until eventually a $4N$ complex is formed to occupy the equatorial coordination sites, when the pH is around 9-10 [15, 17].

4. Specific role of peptides containing gly- residues

Amino acid glycine (Gly, $\text{H}_2\text{N}-\text{CH}_2-\text{COOH}$) is the simplest and smallest among the amino acids found in proteins. It is ambivalent and has aliphatic R-group, Gibbs energies ΔG° are 0.67. In an aqueous solution at or near neutral pH, glycine will exist predominantly as the zwitterions form [18, 19].

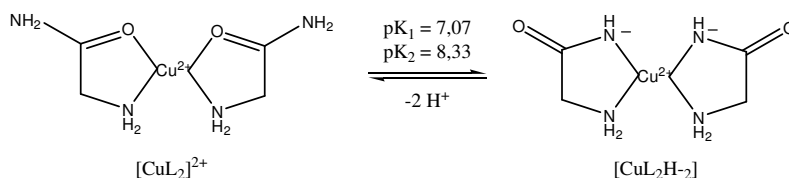
Glycine is the simplest model for peptide coordination, and its complexes with various metal ions have been thoroughly studied. Glycine is of special interest as a model compound for theoretical and experimental studies of the structure and reactivity of amino acids and peptides. According to studies, glycine forms essentially more stable mixed complexes with copper (II) than other amino acids. This phenomenon can also probably be explained by the absence of alkyl chain [18, 19].

The isoelectric point or isoelectric pH of glycine will be centered between the pK_a s of two ionizable groups, the amino group and the carboxylic acid group [10].

Table 1

pK values of glycine oligopeptides [10]

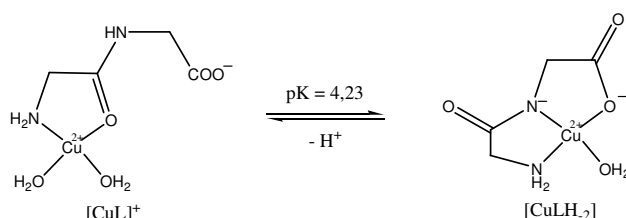
Ligand	$\text{pK}_2 (\text{NH}_3^+)$	$\text{pK}_1 (\text{COOH})$
Glycine	9.60	2.30
Diglycine	8.13	3.21
Triglycine	7.96	3.27
Tetraglycine	7.97	3.24



Coordination modes of glycineamide with Cu(II) ion. The two water molecules weakly coordinated in the axial position are omitted for clarity [20, 21] (3)

In the complexation between oligoglycines and copper (II), the complex formation process involves the successive formation of 1N-, 2N, 3N, or 4N-coordinated species with an increasing pH to form the chelate rings. An example of this complexation is the ability of glycineamide, which forms five-membered chelate rings via the coordination of (NH_2 , CO) donor set (3) [20].

The pK values for amide deprotonation in the Cu(II) complexes of glycineamide are 7.07 and 8.33, lower by at least seven orders of magnitude as compared with the free ligand. The oligoglycines follow a determined system of complexation with Cu(II). The chelation starts at the amino end of the molecule, with the assistance of carbonyl oxygen, and continues with the sequential deprotonation and coordination of the amide groups [20, 21].



Cooper diglycine complexes. The two water molecules weakly coordinated in the axial position are omitted [20, 22] (4)

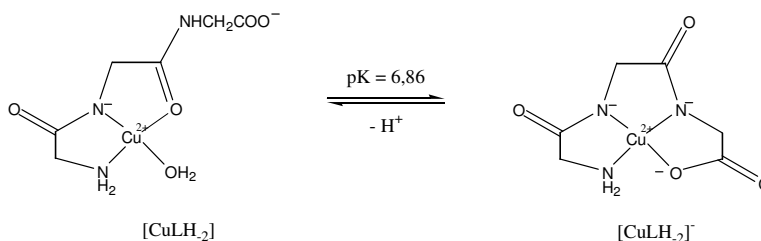
At low pH the dominant species is $[\text{CuA}]^+$, being formed between copper and diglycine with bidentate ligand $[(\text{NH}_2, \text{CO}); \text{H}_2\text{O}; \text{H}_2\text{O}]$ (4). Towards pH 5, as the pK diglycine value is 4.23 (Table 2), the deprotonation of peptidic hydrogens is possible to give another complex species $[\text{CuH}_1\text{L}]$, where H_1 indicates the dissociation of hydrogen; and also the formation of a new five-membered chelate ring. The bonded donor groups to copper are amide-N, the C-terminal carboxylate group via O and the N-terminal amino group via N [10, 22].

Triglycine and tetraglycine continue with the amide deprotonation process and the further formation of extra five-membered chelate rings around Cu(II). $[\text{CuH}_1\text{L}]$ binding groups of triglycine are $[(\text{NH}_2, \text{N}^-, \text{CO}); \text{H}_2\text{O}]$ (5). The pK value of amide deprotonation is 5.41, higher than diglycine (Table 2). When pH increase around the pK₂ of triglycine (6.86), the new conformation will appear $[\text{CuH}_2\text{L}]^-$ [17, 20].

Table 2

pK values for the deprotonation of the amide group in glycine oligopeptides [17, 26, 31]

Ligand	pK ₁	pK ₂	pK ₃
Diglycine	4.23	-	-
Triglycine	5.41	6.86	-
Tetraglycine	5.56	6.91	9.18



Triglycine complexes with Cu(II). Axial water molecules are omitted [17, 20] (5)

The binding ligands of monohydroxylic complex ($[\text{CuH}_2\text{L}]^-$) are $[\text{NH}_2, \text{N}^-, \text{CO}, \text{COO}^-]$. This type of coordination prevents the hydrolytic processes, which occur only in basic solution (pH>12) [17, 20].

Tetraglycine follows the same process but with little differences in the pK values of the amide deprotonation. Tetraglycine can continue the process to form a tetradentate ligand complex which occupies the four equatorial positions of Cu(III) ion with the amino –N and three deprotonated amide-N-. When the pH is around 9, there is again a deprotonation of amide group with the formation of dihydroxo complex or $[\text{CuLH}_3]^{2-}$ [17, 20].

5. Methodology

5.1. Potentiometry

Potentiometry is the field of electroanalytical chemistry in which potential is measured under the conditions of no current flow. The measured potential may then be used to determine the analytical quantity of interest, generally the concentration of some component of the analyte solution [23].

A potentiometer is an instrument for the measurement of electromotive force and also of difference of electric potential between two points. In potentiometry the measuring setup always consists of two electrodes: a measuring electrode, also known as the indicator electrode, and reference electrode. Both electrodes are half-cells. When placed in a solution together they produce a certain potential. Depending on the construction of the half-cells, the potential produced is the sum of several individual potentials. Potential-determining transitions always occur at the phase boundaries, e.g. between the solution and the electrode surface [23].

The reference electrode has constant potential, therefore, no changes have to be provide in this electrode between different experiments. Due to this electrode, any changes in the system potential will be caused by the worker electrode. The measure of the potential is the sum of all the individual potentials generated by the two electrodes. There are different worker electrodes (Glass electrode, Metal electrode, Polymeric membrane electrode, Liquid membrane electrode, Crystal membrane electrode) used for different ions: cations and anions. The most commonly used are the selective electrodes of ions or membrane electrodes. The most often used are the pH electrode and glass electrode [23].

Many types of membranes have been developed (glass, solid state, and even liquid) to detect a wide variety of analytes. In potentiometric pH measurements, an ion selective electrode is used. The potential of such an electrode is usually given by simplified Nernst equation $E = E_0' + 0.0591/n \text{ pa}$ (**E.1**) where n is the charge of an ion and pa is the minus logarithm of its activity. In the case of H^+ ions this equation takes the simplest possible form $E = E_0' + 0.0591 \text{ pH}$ (**E.2**) [23].

It is important to the remember that potential of a pH electrode is measured not as an absolute value, but against the reference electrode of known potential. The most often used reference electrodes are silver chloride electrode and saturated calomel electrode. Depending on the details of pH electrode construction,

a reference electrode can be closed in the same housing or can be separated. This is covered in much more detail in the pH electrode section [23].

The interaction between amino acids, oligopeptides and peptides with metal ions has been explored using electrochemistry methods during years [17, 24].

Speciation, the identification and quantitation of a metal in its various oxidation states, inorganic forms and organometallic complexes, is afforded through a wide variety of techniques as potentiometry [24].

5.2. UV-VIS spectrophotometry

Copper in its various roles in biological systems displays different spectroscopic and chemical properties presumably because of the different ligand environments and coordination numbers [25].

UV-VIS electronic absorption or CD spectroscopies and the magnetic methods including EPR and/or NMR spectroscopies are especially useful techniques to detect various species present in low concentration in solution [25].

Ultraviolet-visible spectrophotometer-molecular absorption spectroscopy in the ultraviolet (UV) and visible (VIS) is concerned with the measured absorption of radiation in its passage through a gas, a liquid or a solid. The generally used wavelength region is from 190 to about 1000 nm, and the absorbing medium is at room temperature; however, in some cases measurements at temperatures above (e.g. in enzyme assays) or below room temperature may be advantageous or necessary [25].

The intensity of light passing through a sample (I) is compared with the intensity of light before it passes through the sample (I_o). The I / I_o rate is called the transmittance, and is usually expressed as a percentage (T). The absorbance, A, is based on the transmittance: $A = -\log(T)$ (E.3). The basic parts of a spectrophotometer are a light source (often an incandescent bulb for the visible wavelengths, or a deuterium arc lamp in the ultraviolet), a holder for the sample, a diffraction grating or monochromator to separate the different wavelengths of light, and a detector. The detector is typically a photodiode or a CCD (Charge Coupled Device). Photodiodes are used with monochromators, which filter the light so that only light of a single wavelength reaches the detector. Diffraction gratings are used with CCDs, which collect light of different wavelengths on different pixels [25, 26].

A spectrophotometer can be either *single beam* or *double beam*. In a single beam instrument, all of the light passes through the sample cell. I_o must be measured by removing the sample. This was the earliest design, but is still in common use in both teaching and industrial labs [25].

In a double-beam instrument, the light is split into two beams before it reaches the sample. One beam is used as the reference; the other beam passes through the sample. Some double-beam instruments have two detectors (photodiodes), and the sample and reference beams are measured at the same time. In other instruments, the

two beams pass through a beam chopper, which blocks one beam at a time. The detector alternates between measuring the sample beam and the reference beam [25].

Samples for UV/Vis spectrophotometry are most often liquids, although the absorbance of gases and even of solids can also be measured. Samples are typically placed in a transparent cell, known as a cuvette. Cuvettes are typically rectangular in shape, commonly with an internal width of 1 cm. (This width becomes the path length, L , in the Beer-Lambert law.) Test tubes can also be used as cuvettes in some instruments. The best cuvettes are made of high quality quartz, although glass or plastic cuvettes are common. (Glass and most plastics absorb in UV, which limits their usefulness to visible wavelengths) [25, 26].

6. Experimental

6.1. Reagents

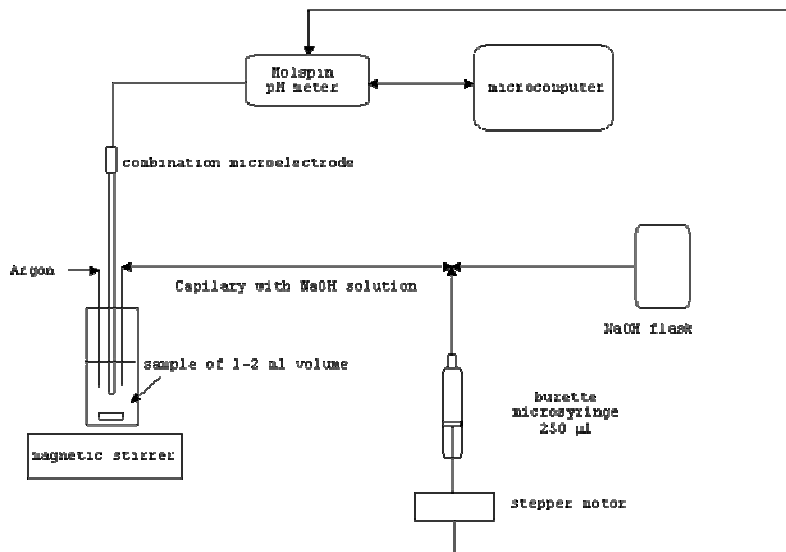
The di-, tri- and tetraglycine peptides were purchased from Bachem and used without purification, $\text{Cu}(\text{NO}_3)_2$, KNO_3 , HNO_3 and NaOH were Merck products and used without any further purification.

6.2. Potentiometric studies

The protonation and coordination equilibria were investigated by potentiometric titration in aqueous solution, over the 2–11 pH range, at constant ionic strength using 0.1M KNO_3 and at constant temperature (298 K) under argon atmosphere with a total volume of 1.5–2 cm³. A 0.04 M solution of $\text{Cu}(\text{NO}_3)_2$ was used as the stock for metal ion. An automatic titration set including autoburette meter (Molspin Ltd., Newcastle-upon-Tyne, UK), a semi-microcombined electrode (Russell CMAWL/S7) and an IBM-compatible PC were used to collect data. Alkali, ca. 0.1M NaOH , free of CO_2 was added from a 0.250 cm³ micrometer syringe, which was calibrated by both weight titration and titration of standard materials. The electrode was calibrated for hydrogen ion activity. The relationship between activity and concentration was calculated daily by titration with HNO_3 . Calculations were made with the aid of SUPERQUAD computer program (6) [27].

This allows the refinement of total ligand concentrations. Therefore it was able to confirm purity of the peptide in particular the absence of acetate, a frequent impurity in peptide samples or other coordinating ions. In all cases triplicate titrations (ca. 500 experimental points in one set of measurements) were carried out at Cu:L ratio 1:1 or 1:2. The metal concentration was 1×10^{-3} mol dm⁻³. As usual, stabilities of the metal complexes are reported as the logarithms of overall formation constants $\beta_{\text{pqr}} = [\text{M}_\text{p}\text{H}_\text{q}\text{L}_\text{r}]/[\text{M}]_\text{p}[\text{H}]_\text{q}[\text{L}]_\text{r}$, where M stands for the metal ion, H is the proton and L the deprotonated form of the ligand (Table 1). The standard deviations quoted were computed by SUPERQUAD and referred to random errors only. They

are, however, a good indication of importance of a particular species in the equilibrium (6) [27].



MOLSPIN apparatus for potentiometric titration

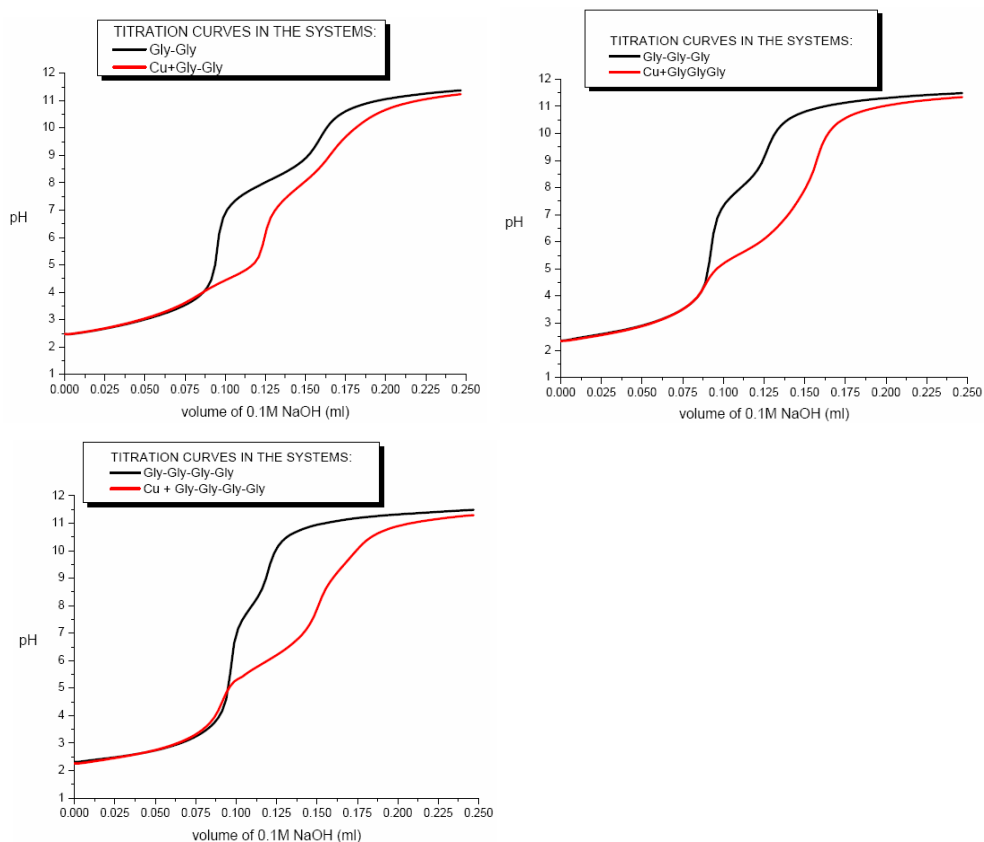
Scheme of MOLSPIN [27] (6)

6.3. Spectroscopic studies

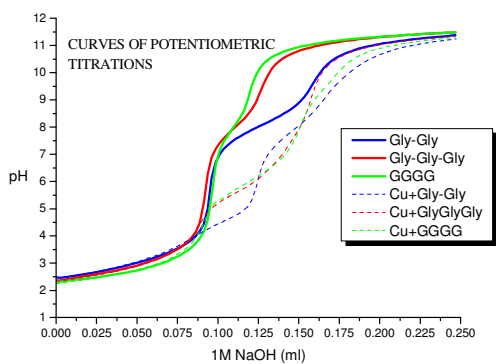
Electronic absorption spectra (UV–Vis) were recorded with Hewlett Packard 8453 spectrophotometer.

7. Discussion and conclusions

The potentiometric titrations were performed in three systems: Cu(II)GG, Cu(II)GGG, and Cu(II)GGGG. As shown in figure (7) and as follows from figure (8), the complexation already starts around pH 3-4.



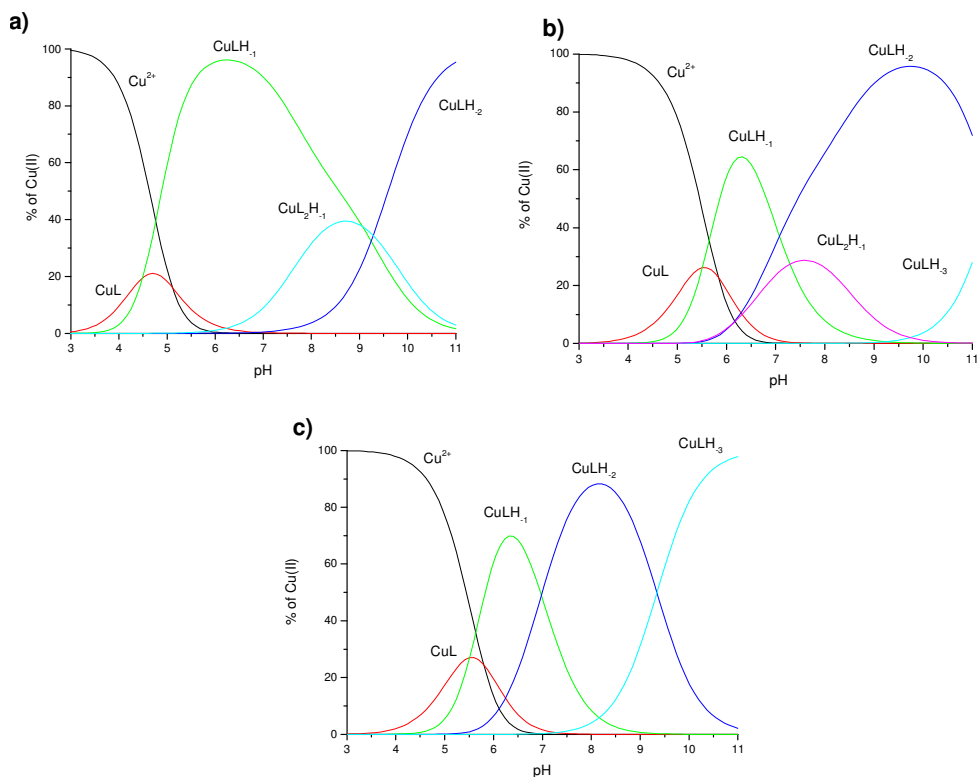
Titration curves in the three system of diglycine, triglycine and tetraglycine (7)



Comparison of titration curves in the three systems: diglycine, triglycine and tetraglycine (8)

Table 3
Formation constants as $\log \beta^a$ (standard deviation of parameter estimate in last valid digits presented in parentheses)
for copper(II) complexes of diglycine, triglycine and tetraglycine

complex	ligand									
	diglycine			triglycine			tetraglycine			
	this work	ref 21	ref 28	this work	ref 21	ref 29	this work	ref 21	ref 30	
HL	8.19(1)	8.13	8.15	8.09(1)	7.93	7.94	7.93(1)	7.94		
H ₂ L	11.38(1)	11.30	11.35	11.23(1)	11.25	11.28	11.30(1)	11.18		
CuL	6.02(1)	5.56	5.63	5.18(3)	5.25	5.08	5.04(3)	5.06	5.07	
CuLH ₋₁	1.53(1)	1.33	1.24	-0.33(2)	-0.16	-0.03	-0.47(2)	-0.50	-0.54	
CuLH ₋₂	-7.71(1)	-8.04	-8.28	-7.41(7)	-7.02	-6.76	-7.45(2)	-7.41	-7.47	
CuLH ₋₃				-18.82(8)	-18.30	-18.66	-16.79(2)	-16.59	-16.78	
CuL ₂ H ₋₁	4.79(1)	4.46	4.34	3.67(4)	3.23					



Species distribution diagrams for the following systems: a) Cu(II)-GG, b) Cu(II)-GGG and c) Cu(II)-GGGG (9)

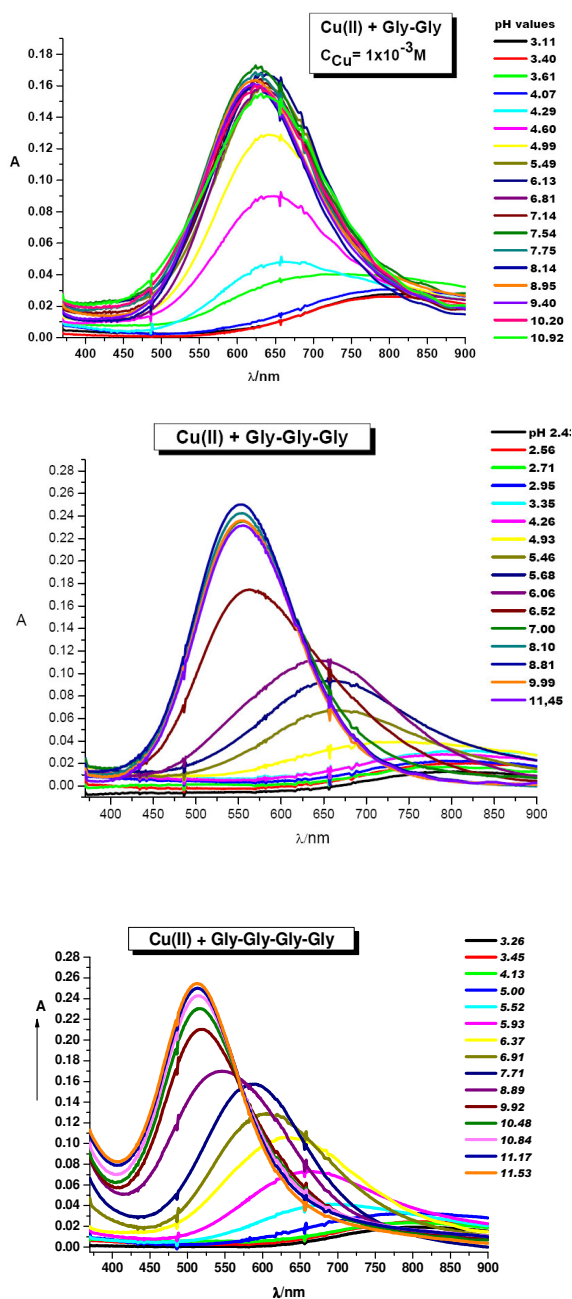
There is a big difference between the graph of diglycine and the other two, triglycine and tetraglycine. The reason of this difference is related to the pK values for the deprotonation of their amide group (**Table 2**). It can be seen that there is a huge leap between the pK of diglycine (pK = 4.23) and the triglycine and tetraglycine (pK = 5.41 and pK = 5.56, respectively).

Also, a little difference in the end of the tri-, tetraglycine graph can be observed. That is caused by the third complexation of tetraglycine which is lacking in triglycine.

The protonation and stability constants for the ligands and complexes have been estimated (**Table 3**). The values of the constants are very close to those in the literature. Some discrepancies occur mainly due to different measurement conditions (e.g. ionic force, concentrations of metal and ligands, etc.). The species distribution diagrams indicate the predominance of a given complex in different pH ranges (9) [17, 31].

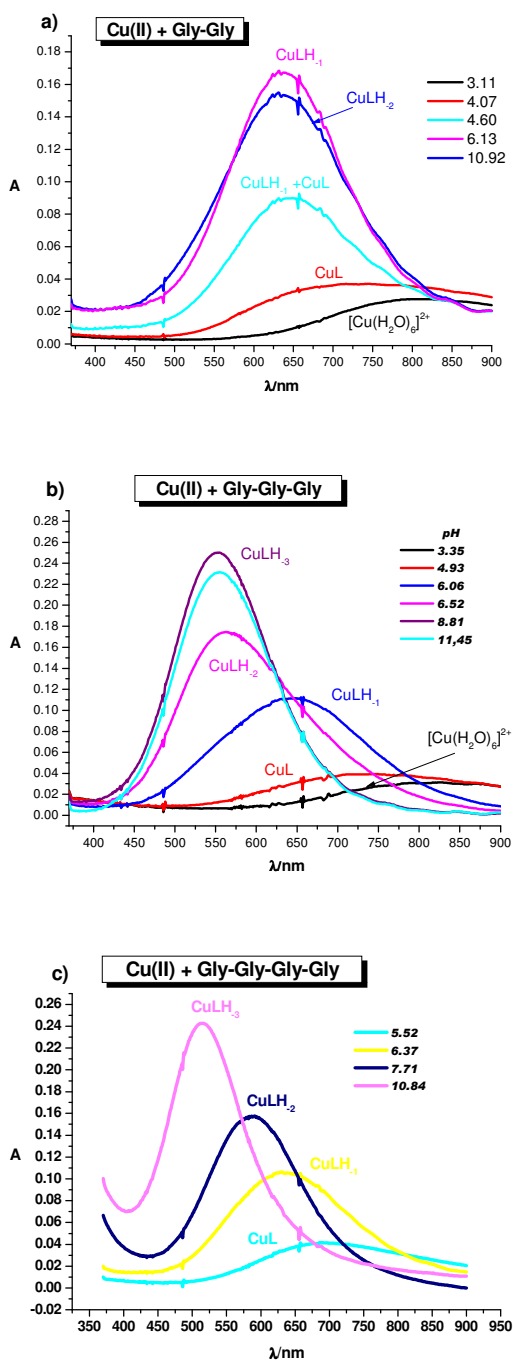
6.4. Spectrophotometric study

The spectrophotometric study of glycine-copper complexes was made also for diglycine, triglycine and tetraglycine systems (10).

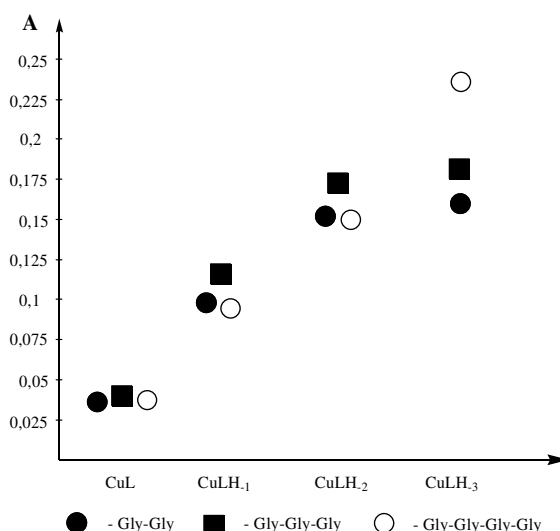


Spectrophotometric curves in diglycine, triglycine and tetraglycine systems (10)

The presence of the main complex species is supported by electronic absorption spectra (11).



Electronic absorption spectra a) diglycine, b) triglycine, c) tetraglycine (II)



Absorption A of various mono complexes, together with the corresponding coordination modes, in copper(II) diglycine, copper(II)-triglycine, and copper(II)-tetraglycine systems (12) [5]

Interesting changes in A can be observed in the series of mono complexes as the numbers of deprotonated peptide groups and coupled chelate rings increase (12). In $[\text{CuL}]^+$, the low value of A corresponds to the sp^3 hybridization state and a moderate bond strength. For the first deprotonation, a significant increase in A indicates the enhanced covalency of copper(II)-N σ -bonds in all $[\text{CuLH}_1]$. For the diglycine complex, there is a significant difference between the two N donors in comparison with the other ligands. We explain this in terms of a different nature of the third donor: the carboxylate group, a strong donor, can bind strongly and stabilize the adjacent copper(II)-peptide N- bond in the diglycine complex, which is manifested in a larger value of the corresponding A . In contrast, the partial coordination of the weak peptide O in the triglycine and tetraglycine complexes has a much weaker effect. The deprotonation of the second peptide NH is accompanied by extremely high values of A , especially for the complex $[\text{CuLH}_2]^-$ of triglycine, and the explanation can be similar to that in the former case. For $[\text{CuLH}_2]^-$ of triglycine, the stabilizing effect of the terminal carboxylate group bound in the fourth position is added, leading to unusually high A values (most probably for the peptide N atoms) (12). For the complex $[\text{CuLH}_3]^{2-}$ of tetraglycine, the third deprotonated peptide group seems to play a similar stabilizing role to that of the carboxylate group in the former species. The above statements can be summarized as follows: when a N atom takes part simultaneously in the formation of two adjacent chelate rings, and two neighbouring donors are bound strongly to the copper(II) ion as well, then the in-plane σ -bond between the N atom and the metal ion becomes particularly strong. Here, we have found a spectroscopic evidence for a significant stabilizing effect in complexes with several coupled chelate rings. It is generally accepted that the water

molecule at the fourth equatorial site of $[\text{CuLH}_1]$ is deprotonated when the mixed hydroxo complex $[\text{CuLH}_2]^-$ of diglycine is formed. The less covalent copper-N σ -bonds seem to be compensated by more covalent π -bonds; a competition between various metal-ligand bonds has been observed for many other copper(II) complexes [31]. Another effect of OH^- is a decrease in the A and the red shift of energy transition (12), which can be explained by 3d—4s orbital mixing as a consequence of a rhombic distortion induced by repulsion between OH^- and the neighboring carboxylate group [32]. For the mixed hydroxo complex $[\text{CuLH}_3]^{2-}$ of triglycine, a small but significant A shift indicates modified equatorial coordination as compared to $[\text{CuLH}_2]^-$. If OH^- replaces the carboxylate group, an increase in A is expected the carboxylate group is not bound in the complexes $[\text{CuLH}_1]$ of triglycine and tetraglycine, than for the corresponding diglycine complex with equatorial carboxylate coordination, and this increase may be counterbalanced in part by the equatorial ligation of OH^- (the latter ligand decreased A in the analogous diglycine complexes). Thus, the increase in A supports the equatorial coordination of OH^- in the complex $[\text{CuLH}_3]^{2-}$ of triglycine, in accordance with the facts that A is smaller for this complex than for the complex $[\text{CuLH}_2]^-$ of tetraglycine, where the fourth equatorial site is partially occupied by a weak peptide O, and the differences in A are much smaller than for the complex $[\text{CuLH}_2]^-$, pointing to a break-down of the stabilization effect of the chelating carboxylate group (12) [31, 32].

7. References

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POTENCJOMETRYCZNE I SPEKTROSKOPOWE BADANIA UKŁADÓW DI-, TRI- I TETRAGLICYNY Z JONAMI MIEDZI (II)

Streszczenie

W pracy tej dokonano przeglądu literaturowego na temat kompleksów jonów miedzi (II) z di-, tri- i tetraglicyną, zwracając uwagę na rodzaje form ich kompleksowania. Przeprowadzono również badania potencjometryczne i spektroskopowe powyższych kompleksów oraz wykazano zależność tworzenia się poszczególnych ich form od pH.

Instytut Chemii Podstaw Żywności
Politechniki Łódzkiej