Transition Metal Complexes of Amino Acids and Derivatives Containing Disulphide Bridges

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Abstract

The interaction of cobalt(II), nickel(II), copper(II) and zinc(II) with D-penicillamine disulphide, oxidized glutathione and L-cysteinylglycine disulphide were studied by pH-metric, spectrophotometric and EPR methods. D-Penicillamine disulphide forms binuclear complexes with all the metal ions studied. The formation of 1:1 complexes is characteristic of oxidized glutathione. L-Cysteinylglycine disulphide behaves like dipeptides, but the presence of two separate peptide moieties also results in the formation of various binuclear complexes. Metal ion–disulphide binding was not observed in any case.

Introduction

Sulphur donor atoms are one of the most important binding sites of metalloproteins, therefore widespread investigations have been carried out to reveal the coordination chemistry of amino acids and peptides containing sulphur donors. Sulphydryl groups generally tightly bind transition metal ions, and their complexation together with possible redox reactions have been extensively studied [1–4]. The replacement of carbonyl oxygen by a sulphur atom in various peptides also significantly increases the donor strength of the ligands, as shown in the investigation of thioamide complexes [5]. On the other hand, the thioether and disulphide groups usually exhibit weak ligation toward 3d-transition metal ions, unless effective neighbouring donor groups are present [6, 7]. Their significance is, however, emphasized by the fact that metal ion thioether or disulphide bonds can be present in various metalloproteins, e.g. in some blue copper–proteins.

D-Penicillamine disulphide (PDS) and oxidized glutathione are the most well-known representatives of disulphide-containing ligands. A copper(II) complex of PDS of composition $(CuPDS)_2$ was prepared by Tich *et al.* [8] and the X-ray structure was determined. In the dimeric complex, copper(II) is coordinated via the amino acid donor groups and a weak axial Cu–S interaction was also suggested. Laurie *et al.* [9] determined stability constants of the copper(II)–PDS system and also found that the dimeric complex is the main species in solution, but the presence of some protonated and bis-complexes was also detected. The equilibrium results of oxidized glutathione are more complicated [10–15]; the formation of 1:1 complexes in which coordination takes place via the glutamic acid residues has been proposed [10, 13–15], but the existence of numerous protonated and various bis-complexes was also suggested [11, 12]. Metal ion–disulphide interaction was not proposed in any system containing oxidized glutathione.

Yamauchi *et al.* [16] studied copper(II) complexes of various disulphide-containing aromatic nitrogen donors and found evidence of direct copper(II)–disulphide interaction in some cases.

In addition to complex formation, redox reactions also can occur with disulphides. It was proved by Musker and Neagley [17] that a mixed valence copper complex of D-penicillamine can be obtained from disulphide and copper(I). On the other hand, disulphides can disproportionate in basic solution which is promoted by metal ions [18].

No equilibrium studies are available on complex formation with simple dipeptides containing disulphide bridges. In this paper we present equilibrium and spectral data for the complexes of L-cysteinylglycine disulphide (CGD) with copper(II), nickel(II), cobalt(II) and zinc(II). Complexes of PDS and oxidized glutathione were also studied for comparison.

Experimental

The chemicals L-cysteinylglycine in the oxidized form (CDG), L-glutathione (oxidized form) (Serva)

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and D-penicillamine disulphide (Aldrich) were used without further purification. The concentrations of metal chloride stock solutions were measured gravimetrically via precipitation of the oxinates.

In the pH-metric measurements the ligand and the metal ion concentrations varied in the range 1×10^{-3} to 5×10^{-3} mol dm^{-3}. Measurements were made at metal ion-to-ligand ratios of 2:1 to 1:4. During titration of the 10 cm^3 samples with CO_2-free KOH, argon was bubbled through them to ensure the absence of atmospheric O_2 and CO_2, and to mix the solutions. Ionic strength was adjusted to 0.2 mol dm^{-3} with KCl. All measurements were made at 25 ± 0.1 °C. Measurements involved the use of a Radiometer pHM84 pH-meter with a GK 2421 C combined electrode and an ABU 80 automatic burette. The method of calculating the $[H^+]$-concentration from the measured pH and the other details of pH-metric procedure were reported earlier [19]. The stability constants (defined by concentrations) were calculated by means of a general equilibrium evaluation program, PSEQUAD [20].

Absorption spectra of copper(II) complexes were recorded on a Beckman Acta MIV spectrophotometer under conditions analogous to those employed for pH-metric measurements. ESR spectra were recorded on a JEOL JES-ME-3X spectrometer at 120 K and 9.14 GHz.

Results and Discussion

pH-metric measurements of the systems have been carried out at various absolute concentrations and metal ion-to-ligand ratios. In this way 150 to 200 experimental data were used to obtain the final stability constants which are collected in Table I–III.

It can be seen from Table I that the process of complex formation of PDS is quite simple because only the complexes of $[MAH]^+$ and $[M_2A_2]$ are formed with all metal ions studied. In the computer evaluation, the monomeric complex [MA] and various bis-complexes ($[MA_2]^{2-}$, $[MA_2H]^-$ and $[MA_2H_2]$) were also taken into account, but their concentrations were negligible and the best fitting was obtained with the species collected in Table I. The corresponding concentration distribution curves for copper(II) complexes are depicted in Fig. 1.

In agreement with previous findings [8, 9] this result again suggests the predominant role of binuclear species with amino acid-like coordination (**I**).

In addition to $[M_2A_2]$, Laurie and coworkers [9] proposed the existence of bis-complexes, mainly in basic solution. However, the interaction of metal ions with PDS is not reversible at pH values above 9, because various transition metal ions promote the following disproportionation of disulphides [18]:

$$2R{-}S{-}S{-}R + 2OH^- = 2RS^- + 2RSOH$$

$$2RSOH = RSH + RSO_2H$$

$$2R{-}S{-}S{-}R + 2OH^- = 2RS^- + R{=}SH + RSO_2H$$

This process occurred with all ligands studied in this work (details of the reactions will be published separately). The existence of disproportionation means that only experimental points below pH 8.5 were used for calculation. Figure 1 shows that $[M_2A_2]$ is the favoured species and its high stability excludes the appearance of various bis-complexes. On the other hand, it should be considered that various spectral studies cannot give an unambiguous proof of the non-existence of bis-complexes because the same amino acid-like coordination is present both in dimeric and in bis-complexes. The results obtained for the other two disulphides, however, support the exclusive formation of dimeric species.

Oxidized glutathione has six dissociable protons of which two belong to the ammonium groups and the other four to the carboxylate groups. The difference between pK values is close to 0.6 log unit suggesting that there is no interaction between the similar donor groups in the big molecule. For the metal ion-containing systems the best fitting was obtained

TABLE I. Stability Constants of Proton and Transition Metal Complexes of D-Penicillamine Disulphide[a]

Species	pK values	Co(II)	Ni(II)	Cu(II)	Zn(II)
$[HA]^-$	8.75 ± 0.03				
$[H_2A]$	16.51 ± 0.03				
$[H_3A]^+$	18.60 ± 0.05				
$[H_4A]^{2+}$	20.14 ± 0.05				
$[MAH]^+$		11.85 ± 0.05	12.90 ± 0.04	15.82 ± 0.03	12.22 ± 0.05
$[M_2A_2]$		12.29 ± 0.04	16.27 ± 0.04	27.97 ± 0.03	14.04 ± 0.05

[a] T = 298 K; I = 0.2 mol dm^{-3}; $pM + qA + rH \rightleftharpoons M_pA_qH_r$; $\beta_{pqr} = \dfrac{[M_pA_qH_r]}{[M]^p[A]^q[H]^r}$

TABLE II. Stability Constants of Proton and Transition Metal Complexes of Oxidized Glutathione[a]

Species	p*K* values	Co(II)	Ni(II)	Cu(II)	Zn(II)
$[HA]^{3-}$	9.90 ± 0.03				
$[H_2A]^{2-}$	18.34 ± 0.03				
$[H_3A]^{-}$	22.16 ± 0.03				
$[H_4A]$	25.32 ± 0.04				
$[H_5A]^{+}$	27.71 ± 0.05				
$[H_6A]^{2+}$	29.50 ± 0.08				
$[MA]^{2-}$		7.12 ± 0.04	9.08 ± 0.03	13.91 ± 0.03	7.60 ± 0.04
$[MAH]^{-}$		13.81 ± 0.06	14.96 ± 0.04	18.11 ± 0.04	13.81 ± 0.05
$[MAH_2]$				21.63 ± 0.05	
$[MAH_3]^{+}$				24.61 ± 0.05	
$[M_2A]$		8.7 ± 0.1	11.06 ± 0.08	16.37 ± 0.05	9.8 ± 0.1

[a]T = 298 K; I = 0.2 mol dm^{-3}.

TABLE III. Stability Constants of Proton and Transition Metal Complexes of L-Cysteinylglycine Disulphide[a]

Species	p*K* values	Co(II)	Ni(II)	Cu(II)	Zn(II)
$[HA]^{-}$	7.29 ± 0.03				
$[H_2A]$	13.59 ± 0.03				
$[H_3A]^{+}$	16.98 ± 0.04				
$[H_4A]^{2+}$	19.60 ± 0.04				
$[MAH]^{+}$		9.47 ± 0.04	9.95 ± 0.03	11.99 ± 0.03	9.61 ± 0.05
$[MA]$		2.93 ± 0.04	3.83 ± 0.03	8.56 ± 0.03	3.56 ± 0.05
$[MAH_{-1}]^{-}$			−4.05 ± 0.05	2.70 ± 0.04	
$[MA_2H_{-1}]^{3-}$				4.85 ± 0.06	
$[M_2AH_{-2}]$				2.85 ± 0.03	
$[M_2A_2H_{-2}]^{2-}$				8.03 ± 0.06	
$[MA_2H_{-2}]^{4-}$			10.17 ± 0.05		

[a]T = 298 K; I = 0.2 mol dm^{-3}.

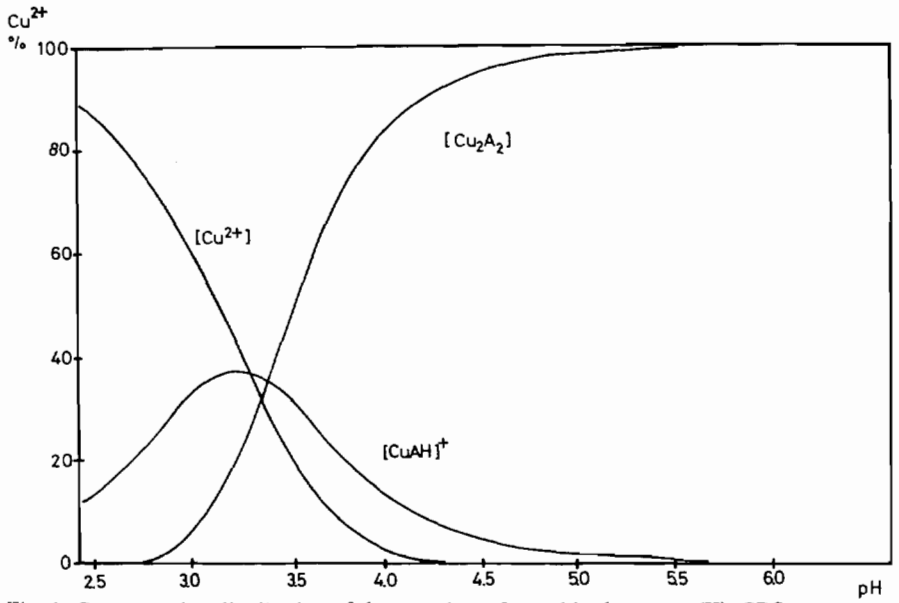
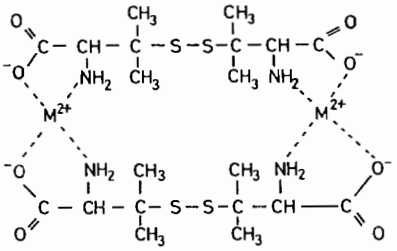


Fig. 1. Concentration distribution of the complexes formed in the copper(II)–PDS system as a function of pH. $C_{PDS} = 4 \times 10^{-3}$; $C_{Cu^{2+}} = 1.33 \times 10^{-3}$ mol dm^{-3}.



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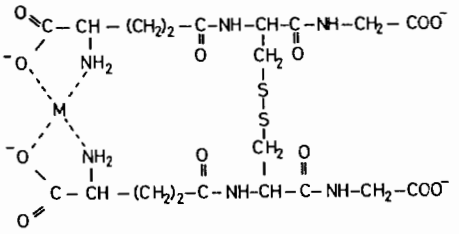


II

with the species collected in Table II. Corresponding concentration distribution curves of copper(II) complexes are depicted in Fig. 2.

From Table II and Fig. 2 it can be seen that the species of composition $[MA]^{2-}$ is the major complex with all metal ions studied, which is in good agreement with the previous findings of Blais and Berthon [14]. The concentration of the dimeric complex $[M_2A]$ is very low in equimolar solutions, but it can be an important species in the presence of excess metal ion. The protonated complex $[MAH]^{-}$ is formed with all metal ions. Corresponding pK values suggest that the glutamylamino group remains – at least in part – protonated; *i.e.* oxidized glutathione binds the metal ions only with half of the molecule via amino acid-like coordination in species $[MAH]^{-}$. Copper(II) complexes of oxidized glutathione are formed at lower pH, where carboxylate groups of glycyl side-chains can be protonated. This results in the existence of further protonated complexes: $[CuAH_2]$ and $[CuAH_3]^{+}$.

In all cases the species $[MA]^{2-}$ is present alone at neutral pH. Its structure can be interpreted by the involvement of the amino acid end in coordination, as shown in **II**.

Absorption spectra of copper(II)–oxidized glutathione in equimolar solutions at pH 7 ($\lambda = 620$ nm; $\epsilon = 61$ mol^{-1} dm^3 cm^{-1}) correspond well to amino acid-like coordination. The 'loop' around the central metal ions contains 19 atoms, which is large enough to give enhanced stability to the $[MA]^{2-}$ species.

Above pH 10 there is a significant spectral change to the lower wavelengths ($\lambda = 585$ nm; $\epsilon = 86.4$ mol^{-1} dm^3 cm^{-1}), which is accompanied by a base consumption process. It is reasonable that amide deprotonation and coordination takes place in that pH range. This fast interaction is, however, overlapped by the slower disulphide hydrolysis, therefore the stoichiometric composition of the complexes cannot be evaluated.

There is no free amino acid side-chain in CGD, therefore steric requirements exclude the formation of a four-coordinated MA complex or binuclear complexes (as in **I** and **II**). The donor groups characteristic of dipeptides, however, are present in this ligand which makes complex formation processes of CGD different from the previous ones.

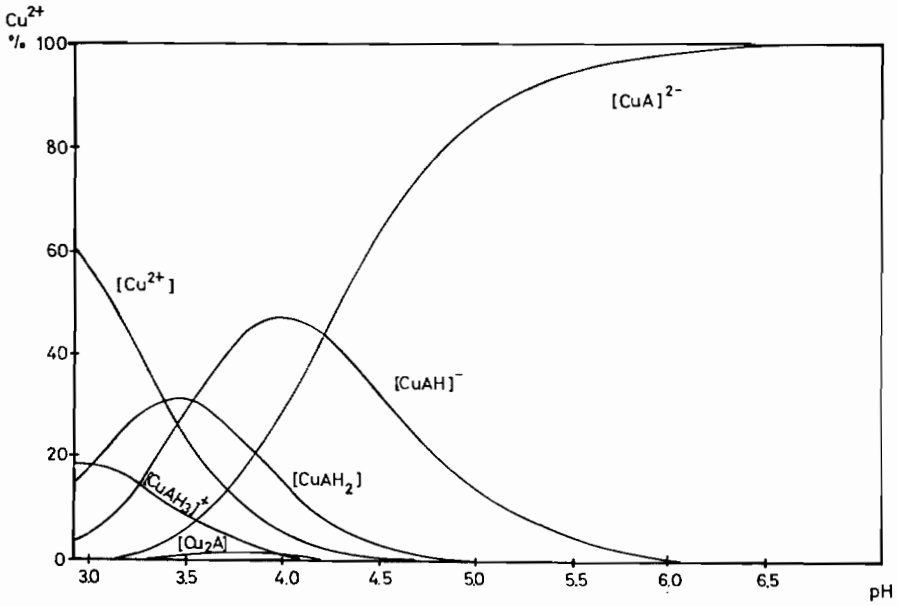


Fig. 2. Concentration distribution of the complexes formed in the copper(II)–oxidized glutathione system as a function of pH. $C_A = 4 \times 10^{-3}$; $C_{Cu^{2+}} = 1.33 \times 10^{-3}$ mol dm^{-3}.

It is well known [21] that cobalt(II) and zinc(II) generally do not induce amide deprotonation and coordination. Consequently, as can be seen from Table III, complexes of [MA] and $[MAH]^+$ are formed only when coordination occurs via amino and neighbouring carbonyl groups. Because of the long side-chain of the molecule, the corresponding stability constants are very low and a significant amount of metal ions can be present in uncomplexed form at the physiological pH range.

Figure 3 shows titration curves for the copper(II)–CGD system.

It can be seen from Fig. 3 that at least two amide groups are deprotonated in one ligand at a ratio of 2:1 for copper(II) and CGD, which can be interpreted by the formation of $[Cu_2AH_{-2}]$. Of course the excess of ligand makes possible the coordination of another CGD molecule which is represented by the species collected in Table III. Figures 4 and 5 show the concentration distribution curves at two different ratios.

From Fig. 4 it can be stated that formation of $[Cu_2AH_{-2}]$ is almost exclusive when an excess of copper(II) is present. The binding mode of the ligand is represented by **III**, and it is supported by visible and EPR spectra.

The absorption maxima of complex $[Cu_2AH_{-2}]$ appear at $\lambda = 635$ nm with $\epsilon = 79.0$ mol^{-1} dm^3 cm^{-1} for total copper(II), which is similar to simple dipeptide complexes. EPR spectra also support the coordi-

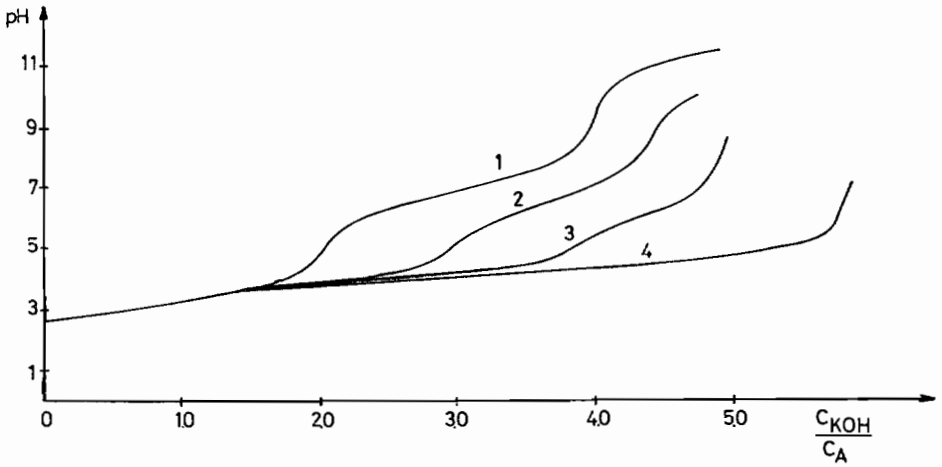


Fig. 3. pH-metric titration curves for the copper(II)–CGD system. $C_{CGD} = 2 \times 10^{-3}$ mol dm^{-3}; $C_M = 0(1); 10^{-3}$ (2); 2×10^{-3} (3); 4×10^{-3} (4) mol dm^{-3}.

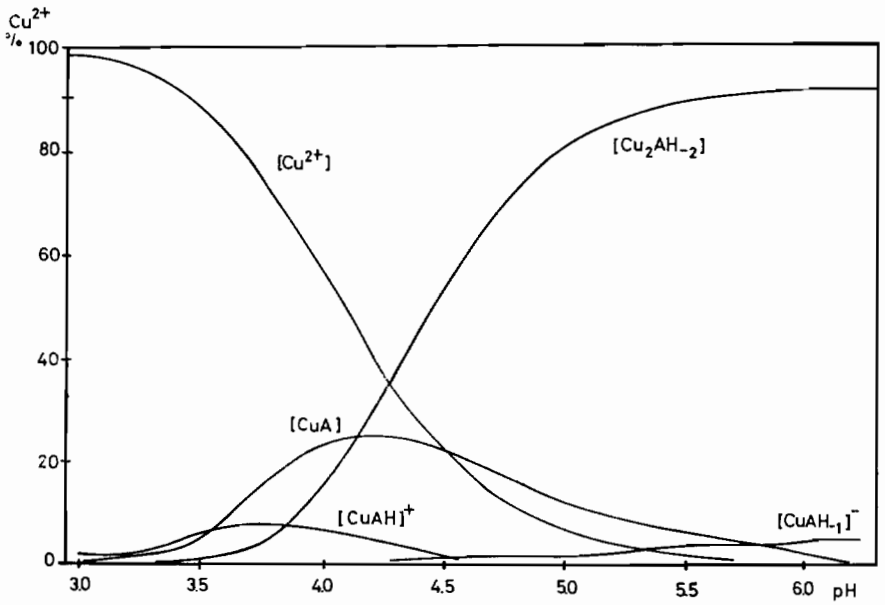


Fig. 4. Concentration distribution of the complexes formed in the copper(II)–CGD system as a function of pH. $C_{CGD} = 2 \times 10^{-3}$; $C_{Cu^{2+}} = 4 \times 10^{-3}$ mol dm^{-3}.

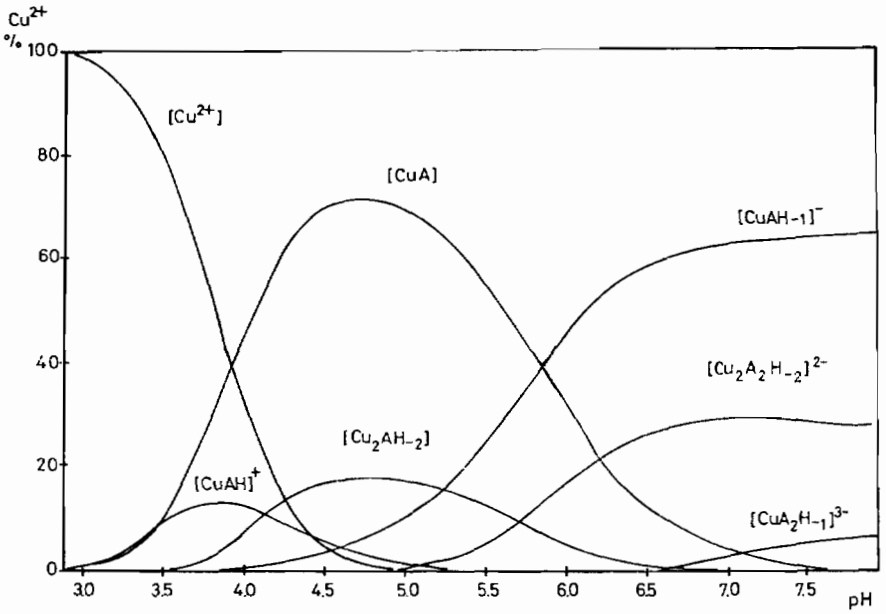
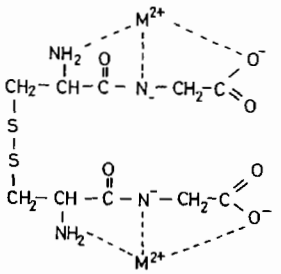


Fig. 5. Concentration distribution of the complexes formed in the copper(II)–CGD system as a function of pH. $C_{CGD} = 2 \times 10^{-3}$; $C_{Cu^{2+}} = 1 \times 10^{-3}$ mol dm^{-3}.



III

nation of all metal ions via the amino, deprotonated amide and carboxylate groups. The copper(II)–copper(II) interaction cannot be observed due to the large distance between the two ends of the molecule.

At pH ~5 the same absorption and EPR spectra can be observed in equimolar solutions or even in the presence of excess ligand. Apparently, this is in contradiction with the concentration distribution depicted in Fig. 5, where [MA] is the main species at pH 5. The spectral data suggest that only one end of the molecule can coordinate to copper (via NH_2, N^- and COO^-), while the other amonium group remains protonated at that pH. In this way the 'real composition' of [CuA] is $[Cu(AH_{-1})H]$ with the same coordination site as in $[Cu_2AH_{-2}]$. A significant blue shift is observed in absorption spectra above pH ~6 (λ = 605 nm) which suggests the coordination of one more nitrogen and it results in the formation of $[Cu_2A_2H_{-2}]^{2-}$ and $[CuA_2H_{-1}]^{3-}$ in which the second ligand is bonded monodentately via the amino group. In other words, the 'real' composition of the complexes is: $[Cu_2A_2H_{-2}]^{2-} = Cu_2(AH_{-2})(A)$ and $[CuA_2H_{-1}]^{3-} = Cu(AH_{-1})(A)$.

Data in Table III reveal that nickel(II) is also able to induce amide deprotonation and coordination which corresponds to results found with other dipeptides [21]. Deprotonation, however, occurs at higher pH than in copper(II), therefore only the complexes of $[NiAH_{-1}]^-$ and $[NiA_2H_{-2}]^{4-}$ can be present.

Summarizing the results for the three different disulphides, it can be stated that the presence of amino acid or peptide linkages is the governing factor for the complexation with transition metal ions. $[M_2A_2]$ binuclear complexes are the main species with PDS, while the long distance between the glutamic acid residues in oxidized glutathione stabilizes the $[MA]^{2-}$ complex. Metal ions are coordinated via amino acid residues in both complexes. Peptide deprotonation and coordination take place in the complexes of CGD with copper(II) and nickel(II), and the ligand behaves as a double dipeptide.

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