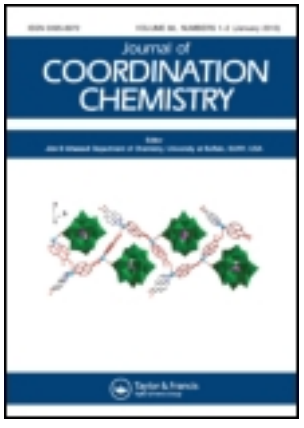

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Complexes of copper(II) with L-aspartic acid in systems with tetramines and non-covalent interactions between bioligands

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In metal-free systems, interactions were studied between L-aspartic acid (Asp) and the tetramines 1,11-diamino-4,8-diazaundecane (3,3,3-*tet*) and 1,12-diamino-4,9-diazadodecane, spermine (*Spm*), and in the systems of both ligands with Cu(II). The formation of molecular complexes of the (Asp)H_x(tetramine) type was evidenced by results from equilibrium and spectral studies. An increase in the efficiency of the reaction between the bioligands was observed with the increasing length of the polyamine chain. For aspartic acid, the centers of interaction are carboxyl and amine groups, whereas in tetramine molecules it is the amine groups. The effect of inversion was observed in the adducts (Asp)H_4(tetramine) and (Asp)H_3(tetramine). In ternary systems, the presence of molecular complexes of ML···L′, protonated complexes MLH_xL', complexes MLL′, and hydroxo complexes of the $MLL'(OH)_x$ type were found, which were not detected in earlier systems with di- and triamines. In ML···L′ complexes, where L = L-aspartic acid and L′ = polyamine, metallation involves the oxygens of carboxyl and amine of the amino acid, while the protonated tetramine is in the external coordination sphere, engaged in noncovalent interaction with the anchoring Cu(Asp). Moreover, in Cu(II)–Asp-tetramine in protonated species, noncovalent bonds were found between ligands which additionally stabilized the complex. At higher pH, hydroxyl groups were more effective in metallation than carboxyl groups from aspartic acid.

Keywords: Copper(II); L-Aspartic acid; Tetramine; Adducts; Mixed complexes; Interaction

1. Introduction

Aspartic acid (Asp) is a naturally occurring α-amino acid with two carboxylic groups in the side chain. This acid is an important neurotransmitter in the central nerve system [1–6]. It is a component of active centers of some enzymes and takes part in thermogenic processes induced by prostaglandin E_1 (PGE_1) [7]. Changes in the concentration of free L-amino acids, including aspartic and glutamic acids in the brain tissue of Alzheimer sufferers with respect to healthy persons, permit evaluation of the degree of degradation of nerve tissue caused by this disease [8–16]. Like amino acids, proteins, and nucleic acids, polyamines (PAs) such as putrescine (*Put*), spermidine (*Spd*), and spermine (*Spm*), are present in all animal cells at high concentrations [17]. Less important are the structural homologues of PAs, for example, norspermine (3,3,3-*tet*), whose level in living cells is much lower. At physiological pH, the amino groups of PAs are fully protonated and interact with negative fragments of other biomolecules, that is, amino acids, transmitter receptor

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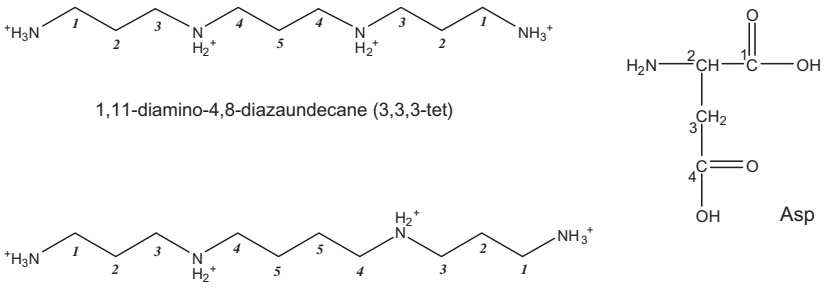

Scheme 1. Chemical formulas of the bioligands studied.

proteins, and phospholipids, playing a significant role in many processes taking place in living cells [18–23]. PAs are involved in cell growth and proliferation, chromatin organization, mRNA translation, synthesis of proteins and nucleic acids, ribosome biogenesis, and programmed cell death [19, 24–28]. Determination of changes in the concentration of PAs in cells (in many diseases from cancer, psoriasis to parasitic infections) and control of their metabolism permits treatment or prevention of certain conditions [29–35]. The main factor determining noncovalent interactions in Asp/PA systems is the high basicity of PA donors [36] forming positive centers of weak interactions that can react with the negative centers of carboxyl groups of amino acids. Interactions of this type have been studied earlier, for example, in the systems of nucleotide/polyamine or nucleotide/Asp, in which these interactions have the character of ion–dipole or ion–ion type [37–44]. Recognition of the nature of interactions in metal-free systems is an important aspect in evaluating the role of metal ions in biological systems. Many questions concerning the role of metal ions, among them transient metal ions, in living organisms are far from being solved [45–49]. Reactions between metal ions and amino acids are models of the processes which take place at the molecular level in the metal/protein system. Copper is an essential trace element required by all living organisms, needed for normal metabolic processes in cells. Among others, it is a cofactor for many enzymes like ascorbate oxidase, amine oxidases, or lysil oxidase and is used as a chemical control agent for micro-organisms [50, 51]. Copper(II) complexes, in particular those in which one ligand is an amino acid, are supposed to be good models of the processes which take place at the molecular level in the metal–protein system [52–55]. Moreover, copper(II) ions play a significant role in Alzheimer's disease by complexing with β-amyloid protein, which is recognized as the main factor causing this condition [56, 57].

This study was aimed at investigating ternary systems that consist of Cu(II) with L-aspartic acid and some tetramines: *Spm* or 3,3,3-*tet* (Scheme 1), and interactions of these biological ligands in metal-free systems in order to characterize the complexes formed and to confirm the unexpected observation of the inversion effect which was detected in analogous systems which included diamines [58] and triamines [59].

2. Experimental

The compounds 1,11-diamino-4,8-diazaundecane (3,3,3-*tet*) – $C_9H_{24}N_4$ and 1,12-diamino-4,9-diazadodecane, *Spm* – $C_9H_{26}N_4$ were purchased from Sigma. The tetranitrates of PAs were prepared by dissolving an appropriate amount of free amine and addition of an equimolar amount of HNO_3. The white precipitate obtained was recrystallized, washed with

methanol and dried in a desiccator over P_4O_{10} or in air. The ligands which were used as nitrate salts were subjected to elemental analysis whose results (%C, %N, %H) were in agreement with the theoretically calculated values (±0.5%). Elemental analyses were performed on an Elemental Analyzer CHN 2400, Perkin-Elmer. L-aspatric acid (Asp) $C_4H_7NO_4$ was bought from Sigma–Aldrich Co. and used without purification. Copper(II) nitrate from POCh – Poland was purified by recrystallization from water. The complexometric method of Cu(II) concentration determination was described earlier [60]. Potentiometric studies were performed on a Methrom 702 SM Titrino with an autoburette. A glass electrode Methrom 6.0233.100 was calibrated in terms of hydrogen ion concentration [61] with borax (pH 9.225) and phthalate (pH 4.002) standard buffers. The concentration of the Asp and tetramines was 1×10^{-2} M in the metal-free systems and from 1×10^{-3} to 2.5×10^{-3} M in the systems with Cu(II). The ratio of L : L′ (where L = aspartic acid and L′ = tetramine) in the metal-free systems was 1 : 1 and in the Cu : L : L′ systems was 1 : 1.8 : 1.8 or 1 : 2.6 : 2.6. Potentiometric titrations were performed at constant ionic strength = 0.1 M (KNO_3), at 20 ± 1 °C under helium, using as a titrant CO_2-free NaOH solution (about 0.2 M). Addition of NaOH solution did not change the ionic strength because the measurements were performed by starting from fully protonated PAs, so that $-NH_x^+$ cations were replaced by equivalent amounts of Na^+. The selection of the models and the determination of the stability constants of the complexes were made using the SUPERQUAD computer program [62]. The calculations were performed using 100–350 points, for each experiment. Distribution of particular forms was determined by the HALTAFALL computer program [63]. Samples for ^{13}C NMR investigation, prepared by dissolving appropriate amounts of the ligands in $D_2O{\cdot}DNO_3$ and NaOD, were used to adjust the pD of the solutions, correcting pH-readings (a pH meter N517 made by Mera-Tronik was used) according to the formula $pD = pH_{readings} + 0.40$ [64]. The concentration of ligand in the samples was 0.05 M and the concentration ratio of Cu(II) to Asp and PA was 1 : 100 : 100. ^{13}C NMR spectra were recorded on an NMR Gemini 300VT Varian spectrometer using dioxane as an internal standard. The positions of ^{13}C NMR signals were converted to the TMS scale. UV–Vis spectra were taken on an Evolution 300 UV/Vis Spectrophotometer Thermo Fisher Scientific for the same ligand concentration as in samples for potentiometric titrations at the metal-to-ligand ratio of 1 : 1.8 : 1.8 and 1 : 2.6 : 2.6 in 1 cm^3 cells. Electron Paramagnetic Resonance spectroscopy (EPR) studies were recorded on an SE/X 2547 Radiopan spectrometer at 77 K in glass capillary tubes of 130 µl capacity. Samples were made up in a water–glycol mixture (3 : 1). The concentration of Cu(II) ions was 5×10^{-3} M and ligands were 9×10^{-3} or 1.3×10^{-2} M.

3. Results and discussion

3.1. *Noncovalent interactions in the Asp/3,3,3-tet and Asp/Spm systems*

In the first stage of the study, interaction between ligands was observed, which was needed to establish the function of metal ions in such systems.

3.1.1. L-Aspartic acid – 3,3,3-*tet* system. Analysis of the potentiometric data collected for the aspartic acid and 3,3,3-*tet* system showed formation of $(Asp)H_6$(3,3,3-*tet*), $(Asp)H_5$(3,3,3-*tet*), $(Asp)H_4$(3,3,3-*tet*), and $(Asp)H_3$(3,3,3-*tet*). The overall stability constants ($\log \beta$) and equilibrium constants for the formation of particular species ($\log K_e$) are given in table 1.

Table 1. Overall stability constants (log β) and equilibrium constants of adduct formation (log K_e) in Asp-3,3,3-*tet* and Asp–*Spm* systems.

Species	Σ	$\chi 2$	Equilibrium	log β	log K_e
(Asp)H_6(3,3,3-*tet*)	41.09	22.09	H_2Asp + $H_4$3,3,3-*tet* ⇆ (Asp)H_6(3,3,3-*tet*)	51.50 (7)	1.75
(Asp)H_5(3,3,3-*tet*)			HAsp + $H_4$3,3,3-*tet* ⇆ (Asp)H_5(3,3,3-*tet*)	48.20 (4)	2.18
(Asp)H_4(3,3,3-*tet*)			HAsp + $H_3$3,3,3-*tet* ⇆ (Asp)H_4(3,3,3-*tet*)	40.95 (3)	2.31
(Asp)H_3(3,3,3-*tet*)			HAsp + $H_2$3,3,3-*tet* ⇆ (Asp)H_3(3,3,3-*tet*)	32.09 (4)	2.08
(Asp)H_6(*Spm*)	31.28	24.65	H_2Asp + H_4*Spm* ⇆ (Asp)H_6(*Spm*)	54.77 (2)	2.74
(Asp)H_5(*Spm*)			HAsp + H_4*Spm* ⇆ (Asp)H_5(*Spm*)	51.08 (2)	2.78
(Asp)H_4(*Spm*)			HAsp + H_3*Spm* ⇆ (Asp)H_4(*Spm*)	42.72 (2)	2.70
(Asp)H_3(*Spm*)			HAsp + H_2*Spm* ⇆ (Asp)H_3(*Spm*)	33.10 (4)	2.19

Overall protonation constants of the ligands: H_3Asp, 15.42; H_2Asp, 13.36; HAsp, 9.63 [44]; $H_4$3,3,3-*tet*, 36.39; $H_3$3,3,3-*tet*, 29.01; $H_2$3,3,3-*tet*, 20.38; H3,3,3-*tet*, 10.36; [65]; H_4*Spm*, 38.67; H_3*Spm*, 30.39; H_2*Spm*, 21.28; H*Spm*, 10.91 [65].

Formation of molecular complexes in the above system (as well as in the Asp/*Spm* system) can be described by the equation:

$$H_xAsp + H_y(3,3,3\text{-}tet) \leftrightarrows (Asp)_{H(x+y-n)}(3,3,3\text{-tet}) + nH^+ \quad (1)$$

Liberation of protons in this reaction permits the use of the potentiometric method for determination of the composition and stability constants (log β) of the adducts. The procedure of iteration allows identification of the types and assessment of the thermodynamic stability of the complexes formed in the systems studied [37, 66, 67].

(Asp)H_6(3,3,3-*tet*) forms at pH below 3, as presented in the distribution of species forming in the system [figure 1(a)]. In the pH range 3–8.5, the dominant species is (Asp)H_5(3,3,3-*tet*) whose maximum concentration at pH 5.5 is close to 45%, and in the pH range 6–10 (Asp)H_4(3,3,3-*tet*) is formed. Above pH 7.5 (Asp)H_3(3,3,3-*tet*) appears and at pH near 9.5 it reaches a maximum concentration of 35%. The noncovalent centers of interaction in the adduct were identified on the basis of NMR results, see table 2. The electron density on atoms neighboring the centers of noncovalent interactions changes as a result of

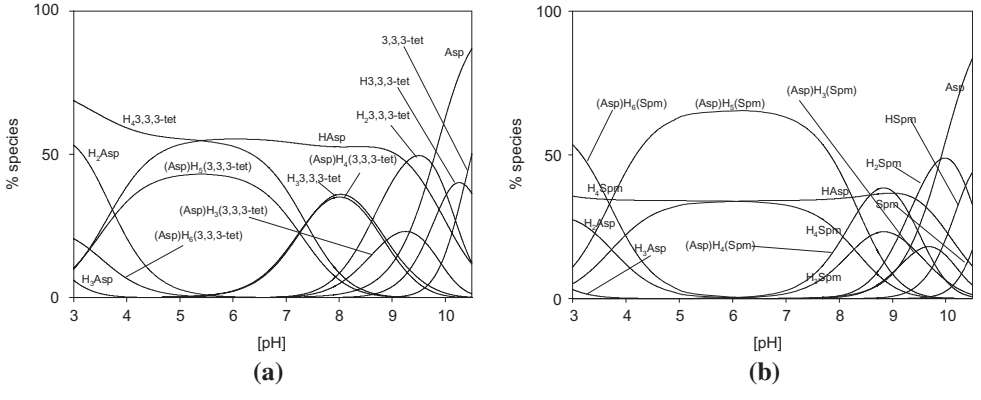

Figure 1. Distribution diagrams for the Asp-3,3,3-*tet* and Asp–*Spm* systems; percentage of the species refers to total Asp (a) Asp-3,3,3-tet: $C_{Asp} = 1.0 \times 10^{-3}$ M; $C_{3,3,3\text{-}tet} = 1.0 \times 10^{-3}$ M; (b) Asp–*Spm*: $C_{Asp} = 1.0 \times 10^{-3}$ M; $C_{Spm} = 1.0 \times 10^{-3}$ M.

Table 2. Differences between ^{13}C NMR chemical shifts for the ligands in the Asp–tetramine systems in relation to the free isolated ligands [ppm].

		Asp				PA				
Systems	pH	$C_{(1)}$	$C_{(2)}$	$C_{(3)}$	$C_{(4)}$	C(1)	C(2)	C(3)	C(4)	C(5)
Asp–3,3,3-*tet*	3.0	0.200	0.053	0.027	0.021	0.131	0.053	0.076	0.070	0.013
	6.0	0.120	0.014	0.073	0.160	0.071	0.052	0.132	0.061	0.094
	8.0	0.408	0.260	0.020	0.134	0.129	0.154	0.043	0.084	0.247
	9.5	0.220	0.214	0.053	0.141	0.097	0.112	0.098	0.097	0.097
Asp–*Spm*	3.0	0.288	0.114	0.041	0.047	0.002	0.041	0.043	0.044	0.060
	6.0	0.101	0.041	0.032	0.081	0.092	0.020	0.040	0.040	0.036
	9.0	0.012	0.121	0.017	0.162	0.085	0.078	0.039	0.040	0.135
	10.0	0.529	0.843	0.202	0.529	0.092	0.093	0.083	0.134	0.226

the interactions, which is observed as a shift in the NMR signals. At pH 3.0, the shifts in the signals assigned to C(1), C(3), and C(4) from PA are 0.131, 0.076, and 0.070 ppm, respectively, while the shift in the signal assigned to $C_{(1)}$ from Asp is 0.200 ppm (table 2). This indicates that in the adduct (Asp) H_6(3,3,3-*tet*), at this pH, the carboxyl group –$C_{(1)}OO^-$ Asp (negative center) and the protonated amines of tetramine (positive centers of interaction) are involved in interactions. The positions of the carbon signals in the ^{13}C NMR spectra of 3,3,3-*tet* and *Spm* were assigned according to the literature [68, 69].

At higher pH, a proton is detached from the second carboxyl group (–$C_{(4)}OO^-$) in Asp and another negative center becomes a potential interaction site in the five-proton adduct. At pH 6, where this complex is dominant, the shifts in the signals assigned to $C_{(1)}$ and $C_{(4)}$ from Asp are 0.120 and 0.160 ppm, while the shift in the $C_{(2)}$ signal in the neighborhood of $-NH_3^+$ of this ligand is only 0.014 ppm. The shifts of signals assigned to C(1), C(3), and C(4) from 3,3,3-*tet* are 0.071, 0.132, and 0.061 ppm, respectively. These changes indicate that oxygens from both carboxyl groups of Asp and protonated amine groups ($-NH_3^+$ and $-NH_2^+$) from 3,3,3-*tet* are involved in noncovalent interactions. The equilibrium constant (log $K_e = 2.18$) for (Asp)H_5(3,3,3-*tet*) is higher than log $K_e = 1.75$ of the (Asp)H_6(3,3,3-*tet*) complex and confirms involvement of the second carboxyl group of Asp in interaction with PA in the five-proton adduct.

(Asp)H_4(3,3,3-*tet*) appears in the pH range in which the first amine of tetramine is already deprotonated [figure 1(a)]. An increase (although small) in the equilibrium constant of this adduct (log $K_e = 2.31$) relative to log $K_e = 2.18$ of (Asp)H_5(3,3,3-*tet*) indicates involvement of another active center in the weak noncovalent interactions. As follows from ^{13}C NMR data, at pH 8, changes in the positions of the carbon signals assigned to C(1), C(3), and C(4) of PA are 0.129, 0.043, and 0.084 ppm, which means that both protonated and deprotonated terminal amine groups from 3,3,3-*tet* are involved in interaction with the amino acid. Shifts in the signals assigned to $C_{(1)}$, $C_{(2)}$, and $C_{(4)}$ from Asp with respect to their positions in the free ligand are 0.408, 0.260, and 0.134 ppm, which means that oxygens from both carboxyl groups and protonated amine $-NH_3^+$ from Asp (inactive at lower pH) are involved in noncovalent interactions with tetramine L···L′ weak interactions). The deprotonated terminal amine 3,3,3-*tet* now becomes a negative reaction center (the partial charges on $-NH_2$, $-NH_2^+$, $-NH_2^+$, and $-NH_3^+$ groups in $H_3$3,3,3-*tet* are −0.033, +0.299, +0.314, and + 0.670, respectively, GAUSSIAN program [70]). It interacts with the protonated amine from Asp being a positive center (the partial charges on the –$C_{(1)}OO^-$, –$C_{(4)}OO^-$, and $-NH_3^+$ groups in HAsp are −0.743, −0.590, and + 0.318 [70]).

Therefore, depending on the pH value, the amine groups from PA can be either positive or negative centers of interaction. Taking into account the fact that all amine groups from 3,3,3-*tet*, and both carboxyl groups and the amine group of Asp, are involved in the interactions as indicated by the NMR results, the intermolecular interaction in (Asp)H_4(3,3,3-*tet*) can be realized only according to the inversion effect, similar to that for (Asp)H_2(tn) [58] and (Asp)H_3(3,3-tri) [59].

At pH above 7.5, at which deprotonation of the second amine from tetramine is noted, (Asp)H_3(3,3,3-*tet*) begins to form. In the ^{13}C NMR spectrum of this complex at pH 9.5, shifts in the signals assigned to $C_{(1)}$, $C_{(2)}$, and $C_{(4)}$ from Asp are 0.220, 0.214, and 0.141 ppm, and those in the signals assigned to C(1), C(3), and C(4) from tetramine are 0.097, 0.098, and 0.097 ppm, respectively. Analysis of the NMR spectra indicates involvement of oxygens from both carboxyl groups and protonated amine from Asp in the interactions of the ligands. The deprotonated primary amine $-NH_2$ (negative centers) and protonated secondary amine groups $-NH_2^+$ (positive centers) from the 3,3,3-*tet* molecule are involved in interaction with Asp. Thus, also in (Asp)H_3(3,3,3-*tet*), the effect of inversion is noted, similarly as in (Asp)H_4(3,3,3-*tet*). However, the equilibrium constant of formation of this species log $K_e = 2.08$ is lower than those of four- and five-proton adducts formed at lower pH (table 1), which means that all available active centers do not take part simultaneously in the weak noncovalent interactions of the ligands.

3.1.2. L-aspartic acid–*Spm* system. Similar to the system with 3,3,3-*tet*, the process of complexation with *Spm* begins with deprotonation of the first carboxyl group of Asp. *Spm* forms with Asp a series of protonated molecular complexes of the $LL'H_x$ type, where $x = 3$–6 [figure 1(b)], whose concentrations are higher than those of the corresponding complexes with 3,3,3-*tet*, which corresponds well to the differences in the equilibrium constants of formation, log K_e (table 1). It confirms that the length of the polyamine chain is important in complex stability, as observed also for Asp/diamine [58] and Asp/triamine systems [59].

At pH 3 in the ^{13}C NMR spectrum of (Asp)H_6(*Spm*), the shifts in the signals assigned to C(3) and C(4) from near nitrogen donors in *Spm* are 0.043 and 0.044 ppm, while the shift of the carbon signal assigned to $-C_{(1)}OO^-$ from Asp is 0.288 ppm (table 2). These results indicate that $-NH_2^+$ from *Spm* and deprotonated $-C_{(1)}OO^-$ from Asp are involved in the interaction. The shift in the signal assigned to C(1) from tetramine of only 0.002 ppm proves that the terminal $-NH_3^+$ from *Spm* are ineffective in the noncovalent interactions in the systems studied. Relatively small but systematic shifts in the positions of the signals are related to the type of interaction.

With increasing pH and deprotonation of the second carboxyl $-C_{(4)}OO^-$ of Asp, the penta-protonated adduct is formed. Analysis of the ^{13}C NMR spectrum at pH 6 reveals shifts in the signals assigned to $C_{(1)}$ and $C_{(4)}$ from Asp by 0.101 and 0.081 ppm, respectively, which points to the involvement of oxygens from both carboxyl groups of Asp in noncovalent interactions. As follows from shifts in the signals of C(1), C(3), and C(4) from *Spm* (table 2), the terminal $-NH_3^+$ froups are the main centers of interactions. Moreover, the log $K_e = 2.78$ of (Asp)H_5(*Spm*) is similar to that of the (Asp)H_6(*Spm*) complex (log $K_e = 2.74$), which suggests a similar mode of interaction in these two adducts.

Above pH 7, when the first proton is dissociated from the amine group of *Spm*, the (Asp)H_4(*Spm*) species begins to form, log $K_e = 2.70$. In the ^{13}C NMR spectrum, the changes in the chemical shifts of the *Spm* C(1), C(3), and C(4) are 0.085, 0.039, and

0.040 ppm, respectively, proving that in the interaction with Asp, as in (Asp)H_5(*Spm*), the terminal amine group from tetramine take part,that is, the deprotonated –NH_2 as a negative center and the protonated $-NH_3^+$ as a positive center. The shifts in the signals are assigned to $C_{(4)}$ from Asp at pH 9.0 is 0.162 ppm, while that of $C_{(1)}$ is only 0.012 ppm. Therefore, only one carboxyl group of this ligand $-C_{(4)}OO^-$ takes part in the interaction, similarly as for (Asp)H_3(3,3-tri) [59]. This observation suggests that dissociation of the first proton from PA can exclude oxygen of the carboxyl group $-C_{(1)}OO^-$ of Asp from interactions. Moreover, the shift in the signal assigned to $C_{(2)}$ of amino acid is 0.121 ppm, which means that the $-NH_3^+$ of Asp (as a positive center, partial charge on this group is + 0.318 [70]) is involved in interactions with the deprotonated terminal –NH_2 from *Spm* (a change in the chemical shift of $C_{(1)}$ is 0.085 ppm), as a negative center now (partial charge on this group is −0.044 [70]). Therefore, from analysis of changes in the ^{13}C NMR signals and partial change on the groups which participate in interaction, similarly as in the corresponding system with 3,3,3-*tet*, inversion in the mode of interaction was concluded in the (Asp)H_4(*Spm*) complex.

In the pH range in which deprotonation of the second amine of tetramine takes place [figure 1(b)], (Asp)H_3(*Spm*) appears. Analysis of the ^{13}C NMR spectrum at pH 10 (table 2) shows that in (Asp)H_3(*Spm*) all available centers of interaction are involved in the interaction. The protonated amine group $-NH_3^+$ from Asp interacts as a positive center with the deprotonated terminal amine group from *Spm* as negative centers, while the $-NH_2^+$ of tetramine interact with oxygens from carboxyl groups of Asp. These results confirm that the inversion effect is also observed in (Asp)H_3(*Spm*). However, a decrease in the equilibrium constant of the (Asp)H_3(*Spm*) adduct, log K_e = 2.19 (similar to that of (Asp)H_3(3,3,3-*tet*)), relative to log K_e values of the other adducts in this system (table 1), suggests that not all active centers take part in the interactions simultaneously.

3.2. *Ternary Cu(II)–L-aspartic acid–tetramine systems*

In the ternary system Cu(II)–Asp–tetramine, determination of stability constants was performed taking into account the earlier obtained protonation constants of the ligands (table 1) and overall stability constants (log β) of complexes forming in the binary systems Cu(II)–Asp [44], Cu(II)–3,3,3-*tet* [65] and Cu(II)–*Spm* [65] (table 3). Moreover, the literature values for the hydrolysis constants of the Cu(II) ions were used [71].

3.2.1. Cu(II)–L-aspartic acid–3,3,3-*tet* system. The computer analysis (SUPERQUAD program) of the potentiometric titration data for the Cu(II)–Asp–3,3,3-*tet* system revealed the presence of the following protonated complexes: Cu(Asp)H_4(3,3,3-*tet*), Cu(Asp)H_3(3,3,3-*tet*), Cu(Asp)H_2(3,3,3-*tet*), a mixed complex Cu(Asp)(3,3,3-*tet*), and the hydroxo complexes Cu(Asp)(3,3,3-*tet*)(OH) and Cu(Asp)(3,3,3-*tet*)$(OH)_2$ that do not form in the systems with shorter chain PAs [58, 59]. Table 3 presents the values of the overall stability constants and equilibrium constants for formation of Cu(II) complexes with Asp and 3,3,3-*tet*. Cu(Asp)H_4(3,3,3-*tet*) occurs in pH range 3–5.5 [figure 2(a)], in which tetramine is fully protonated. The equilibrium constant for Cu(Asp)H_4(3,3,3-*tet*) formation, log K_e = 4.16 (table 3), is similar to the logK_e values of ML···L′ forming in the analogous systems with di- and triamines [58, 59], which points to a similar interaction in these complexes, that is, protonated PA is in the outer coordination sphere and takes part in weak noncovalent interactions with the anchoring binary complexes of copper(II) with the

Table 3. Overall stability constants (log β), statistic parameters (Σ, χ^2) and equilibrium constants (log K_e) of complexes formation in Cu(II)–Asp-3,3,3-*tet* and Cu(II)–Asp–*Spm* systems.

Species	Σ	$\chi 2$	log β	Equilibrium of formation*	log K_e
Cu(Asp)H_4(3,3,3-*tet*)	5.74	15.70	49.30(4)	$Cu^{2+} + 4H^+ + Asp + 3,3,3\text{-}tet \leftrightarrows Cu(Asp)H_4(3,3,3\text{-}tet)^{6+}$	4.16
Cu(Asp)H_3(3,3,3-*tet*)			45.00(5)	$Cu^{2+} + 3H^+ + Asp + 3,3,3\text{-}tet \leftrightarrows Cu(Asp)H_3(3,3,3\text{-}tet)^{5+}$	7.23
Cu(Asp)H_2(3,3,3-*tet*)			39.56(7)	$Cu^{2+} + 3H^+ + Asp + 3,3,3\text{-}tet \leftrightarrows Cu(Asp)H_2(3,3,3\text{-}tet)^{4+}$	10.42
Cu(Asp)(3,3,3-*tet*)			26.75(7)	$Cu^{2+} + Asp + 3,3,3\text{-}tet \leftrightarrows Cu(Asp)(3,3,3\text{-}tet)^{2+}$	17.99
Cu(Asp)(3,3,3-*tet*)(OH)			17.28(10)	$Cu^{2+} + H_2O + Asp + 3,3,3\text{-}tet \leftrightarrows Cu(Asp)(3,3,3\text{-}tet)(OH) + H^+$	–
Cu(Asp)(3,3,3-*tet*)$(OH)_2$			7.17(9)	$Cu^{2+} + 2H_2O + Asp + 3,3,3\text{-}tet \leftrightarrows Cu(Asp)(3,3,3\text{-}tet)(OH)_2 + 2H^+$	–
Cu(Asp)H_4(*Spm*)	7.04	15.94	50.58(4)	$Cu^{2+} + 4H^+ + Asp + Spm \leftrightarrows Cu(Asp)H_4(Spm)^{6+}$	3.15
Cu(Asp)H_3(*Spm*)			45.40(5)	$Cu^{2+} + 3H^+ + Asp + Spm \leftrightarrows Cu(Asp)H_3(Spm)^{5+}$	6.25
Cu(Asp)H_2(*Spm*)			38.00(6)	$Cu^{2+} + 2H^+ + Asp + Spm \leftrightarrows Cu(Asp)H_2(Spm)^{4+}$	7.96
Cu(Asp)H(*Spm*)			29.93(6)	$Cu^{2+} + H^+ + 2Asp + Spm \leftrightarrows Cu(Asp)H(Spm)^{3+}$	10.26
Cu(Asp)(*Spm*)			20.16(7)	$Cu^{2+} + Asp + Spm \leftrightarrows Cu(Asp)H(Spm)^{2+}$	11.40
Cu(Asp)(*Spm*)(OH)			10.31(6)	$Cu^{2+} + H_2O + Asp + Spm \leftrightarrows Cu(Asp)(Spm)(OH) + H^+$	–

*Overall stability constants (log β) of complexes formation in binary systems: Cu(II)-Asp; CuH(Asp), 15.35; Cu(Asp), 8.76; $Cu(Asp)_2$, 12.78; Cu(Asp)(OH), 0.79 (9) [44]; Cu(II)-3,3,3-*tet*: CuH_2(3,3,3-*tet*), 27.49; Cu(H3,3,3-*tet*)$_2$, 42.03; Cu(3,3,3-*tet*), 16.36 [65]; Cu(II)-*Spm*: CuH_2(*Spm*), 27.63; $CuH(Spm)_2$, 29.32; Cu(*Spm*), 14.66 [65].

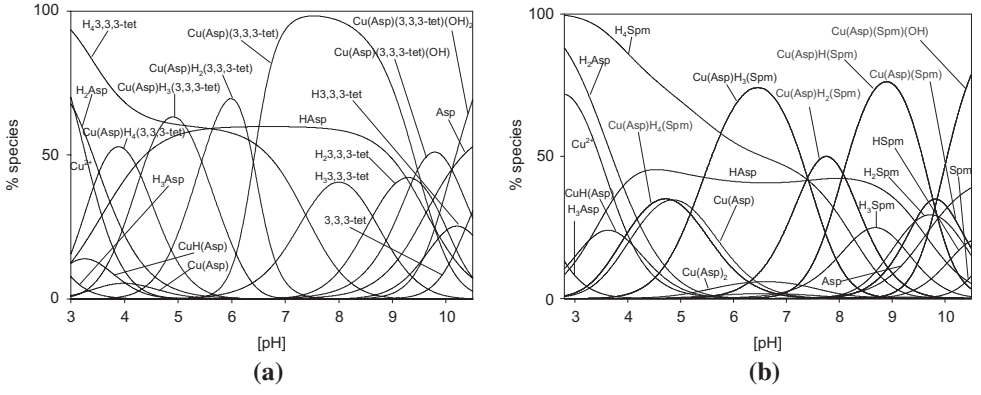

Figure 2. Distribution diagrams for the Cu(II)–Asp-3,3,3-*tet* and Cu(II)–Asp–*Spm* systems; percentage of the species refers to total Cu^{2+} (a) Cu(II)–Asp-3,3,3-*tet*: $C_{Cu2+} = 3.8 \times 10^{-4}$ M; $C_{Asp} = 1.0 \times 10^{-3}$ M; $C_{3,3,3\text{-}tet} = 1.0 \times 10^{-3}$ M; (b) Cu(II)–Asp–*Spm*: $C_{Cu2+} = 5.6 \times 10^{-4}$ M; $C_{Asp} = 1.0 \times 10^{-3}$ M; $C_{Spm} = 1.0 \times 10^{-3}$ M.

amino acid. The metallation sites in the above species were identified on the basis of spectroscopic studies (table 4). The results of UV–Vis ($\lambda_{max} = 746$ nm) and EPR ($g_{||} = 2.259$ and $A_{||} = 167$) spectra obtained at pH 4, at which Cu(Asp)H_4(3,3,3-*tet*) is dominant, point to metal coordination by oxygens from carboxyl groups and nitrogen from the amino acid, thus $\{N,O_x\}$ coordination. Conclusions concerning the mode of coordination were drawn on the basis of analysis of the relation between the energy of the d–d transition as well as EPR parameters and the number of donors in the inner sphere of the Cu(II) complexes. For tetragonal and square pyramidal species, the ground state is normally $d_{x^2-y^2}$ or rarely d_{xy}. As earlier established for Cu–N_x ($x = 1$–6) and Cu–N_xO_y ($x = 0$–4, $y = 0$–6) in planar bonding, the value of $g_{||}$ decreases and that of $A_{||}$ increases [72, 73]. The mode of interaction in Cu(Asp)H_4(3,3,3-*tet*) was confirmed by ^{13}C NMR data. At pH 4, the shifts in the positions of the signals assigned to $C_{(1)}$, $C_{(4)}$, and $C_{(2)}$ from Asp are 0.180, 0.343, and 0.457 ppm, respectively, whereas the shifts in the signals assigned to C(1), C(3), and C(4) from PA are 0.011, 0.166, and 0.085 ppm, respectively (table 5), and indicate (in correlation with the Vis, EPR, and equilibrium results) participation of the fully protonated 3,3,3-*tet* molecule in weak noncovalent interactions with the anchoring binary complex of Cu(II) ions with Asp.

Table 4. Vis and EPR spectral data for Cu(II)/Asp/3,3,3-*tet* and Cu(II)/Asp/*Spm* systems.

				EPR	
Species	pH	λ_{max} [nm]	ε [M^{-1} cm^{-1}]	$g_{||}$	$A_{||}$ [10^{-4} cm^{-1}]
Cu(Asp)H_4(3,3,3-*tet*)	4.0	746	11.8	2.259	167
Cu(Asp)H_3(3,3,3-*tet*)	5.0	708	23.2	2.258	178
Cu(Asp)H_2(3,3,3-*tet*)	6.0	620	33.7	2.251	186
Cu(Asp)(3,3,3-*tet*)	8.0	595	88.4	2.212	184
Cu(Asp)(3,3,3-*tet*)(OH)	9.8	594	88.2	2.220	186
Cu(Asp)(3,3,3-*tet*)$(OH)_2$	10.5	591	86.2	2.225	182
Cu(Asp)H_4(*Spm*)	4.5	743	17.9	–	–
Cu(Asp)H_3(*Spm*)	6.5	645	19.9	2.252	184
Cu(Asp)H_2(*Spm*)	7.8	604	36.2	2.230	198
Cu(Asp)H(*Spm*)	8.8	581	44.9	2.221	197
Cu(Asp)(*Spm*)(OH)	10.5	565	63.8	2.218	194

Table 5. Differences between ^{13}C NMR chemical shifts for the ligands in the Cu(II)-Asp-3,3,3-*tet* or *Spm* systems in relation to the free ligands [ppm].

		Asp				PA				
Systems	pH	$C_{(1)}$	$C_{(2)}$	$C_{(3)}$	$C_{(4)}$	C(1)	C(2)	C(3)	C(4)	C(5)
Cu(II)–Asp–3,3,3-*tet*	4.0	0.180	0.457	0.161	0.343	0.011	0.042	0.166	0.085	0.055
	5.0	0.230	0.190	0.070	0.230	0.097	0.024	0.112	0.141	0.003
	6.0	0.093	0.100	0.086	0.125	0.074	0.012	0.101	0.075	0.025
	8.0	0.521	0.218	0.158	0.748	1.293	1.000	0.357	1.442	1.061
	9.8	3.251	0.266	0.031	0.086	0.220	0.505	0.099	0.073	0.184
	10.5	0.095	3.805	0.142	0.480	0.021	0.010	0.048	0.030	0.089
Cu(II)–Asp–*Spm*	4.5	0.132	0.218	0.037	0.105	0.040	0.040	0.030	0.030	0.040
	6.0	0.442	0.746	0.148	0.765	0.440	0.337	0.089	0.241	0.329
	7.0	0.246	0.230	0.066	0.237	0.110	0.096	0.030	0.025	0.078
	8.8	0.082	0.878	–	–	0.601	0.018	0.630	0.722	0.464
	10.5	1.512	0.504	–	0.287	0.928	1.043	0.910	0.899	0.839

The controversial problem of reliability of NMR measurements in systems with paramagnetic ions has been discussed earlier and the conclusion was that assuming a critical approach it is possible to draw conclusions on the mode of coordination on the basis of analysis of changes in the signal positions, as these changes were significant only in the pH ranges of formation of given complexes whose composition was established from potentiometric titration. The NMR method has already been applied in analysis of similar Cu(II) ion systems [42,44,58,59,74]. To minimize the effect of NMR signal broadening because of the paramagnetic character of Cu(II) ions, the spectra were measured only at their low concentrations (the pH ranges of the complex dominance in the distribution curves of the species are practically the same as for systems of higher concentrations of the metal ions and the ligands). The mode of interactions in the molecular complex Cu(Asp)H_4(3,3,3-*tet*) that is, ML$\cdots$L′ is strongly confirmed by the results of experimental analysis of changes in the d–d band. At pH 4, introduction of increasing amounts of 3,3,3-*tet* to the binary system Cu(II)–Asp does not lead to significant changes in the position of the maximum absorption [figure 3(a)], similarly to system with *Spm*. On the other hand, introduction of aspartic acid to the binary system Cu(II)–3,3,3-*tet* leads to a significant shift in the d–d band assigned to Cu(II) [figure 3(b)], which proves that under the conditions studied, in the Cu(II)–Asp–3,3,3-*tet* system, copper(II) ions are coordinated by donors from Asp (the PA is located in the outer coordination sphere and is not involved in metal bonding, the ML$\cdots$L′ type complex is formed).

With increasing pH and deprotonation of the first amine from 3,3,3-*tet*, Cu(Asp)H_3(3,3,3-*tet*) begins to form. The position of the absorption band in the UV–Vis spectrum at $\lambda_{max} = 708$ nm at pH 5, where this species dominates and the EPR parameter values $g_{\|} = 2.258$ and $A_{\|} = 178$ (table 4) indicate the $\{N,O_x\}$ chromophore. The shifts in the signals assigned to $C_{(1)}$, $C_{(4)}$, and $C_{(2)}$ from *Asp* at pH 5 are 0.230, 0.230, and 0.190 ppm, while those of signals assigned to C(1), C(3), and C(4) from PA neighboring the amine groups of 3,3,3-*tet* are 0.097, 0.112, and 0.141 ppm, respectively. The equilibrium constant of this species formation informs about the effectiveness of interaction of H_3(3,3,3-*tet*) with Cu(Asp). The K_e value is calculated as $\log K_e = \log \beta_{Cu(Asp)H3(3,3,3\text{-}tet)} - \log \beta_{Cu(Asp)} - \log \beta_{H3(3,3,3\text{-}tet)} = 45.00 - 8.76 - 29.01 = 7.23$, and is higher than $\log K_e = 5.08$ for CuH(tn) [65] or $\log K_e = 5.0$ for CuH(Put) [65] (chromophore {N}), which points to an additional stabilization in Cu(Asp)H_3(3,3,3-*tet*). Thus, in this complex, copper(II) ions are

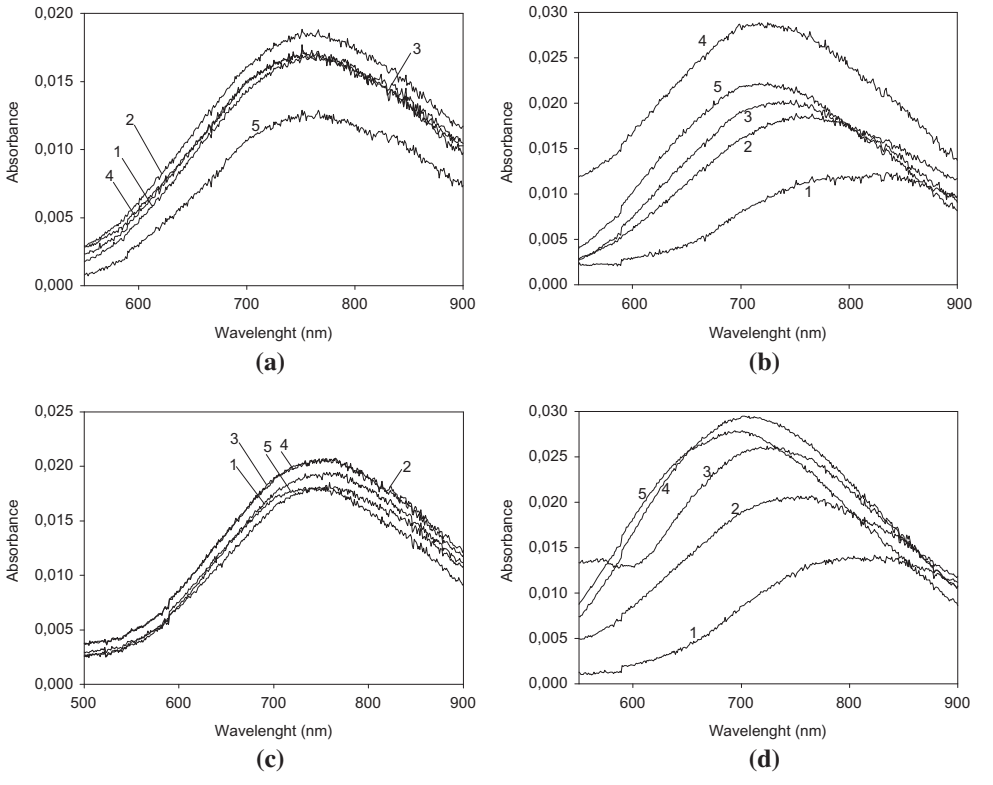

Figure 3. Vis spectra of Cu(II)–Asp–3,3,3-*tet* (pH 4) and Cu(II)–Asp–*Spm* (pH 4.5) systems; $C_{Cu2+} = 1 \times 10^{-2}$ M; (a) 1). Cu(II)/Asp, 2). Cu(II)/Asp/3,3,3-*tet* 1 : 1 : 1, 3). Cu(II)/Asp/3,3,3-*tet* 1 : 1 : 2, 4). Cu(II)/Asp/3,3,3-*tet* 1 : 1 : 3, 5). Cu(II)/Asp/3,3,3-*tet* 1 : 1 : 4; (b) 1). Cu(II)/3,3,3-*tet* 1 : 1, 2). Cu(II)/Asp/3,3,3-*tet* 1 : 1 : 1, 3). Cu(II)/Asp/3,3,3-*tet* 1 : 2 : 1, 4). Cu(II)/Asp/3,3,3-*tet* 1 : 3 : 1, 5). Cu(II)/Asp/3,3,3-*tet* 1 : 4 : 1; (c) 1). Cu(II)/Asp, 2). Cu(II)/Asp/*Spm* 1 : 1 : 1, 3). Cu(II)/Asp/*Spm* 1 : 1 : 2, 4). Cu(II)/Asp/*Spm* 1 : 1 : 3, 5). Cu(II)/Asp/*Spm* 1 : 1 : 4 and (d) 1). Cu(II)/*Spm* 1 : 1, 2). Cu(II)/Asp/*Spm* 1 : 1 : 1, 3). Cu(II)/Asp/*Spm* 1 : 2 : 1, 4). Cu(II)/Asp/*Spm* 1 : 3 : 1, 5). Cu(II)/Asp/*Spm* 1 : 4 : 1.

coordinated by nitrogen from deprotonated $-NH_2$ of PA and oxygens from carboxyl groups of Asp, while protonated amine groups from tetramine take part in noncovalent interactions that further stabilize the complex. Interactions of this type have been observed in complexes of Cu(II) with Asp and *CMP* [44] or Cu(II) with Asp and *tn* [58] systems.

With deprotonation of the second amine group of PA, $Cu(Asp)H_2$(3,3,3-*tet*) begins to form. This species at pH close to 6.0 binds almost 70% of Cu(II) ions [figure 2(a)]. The UV–Vis spectrum of this species shows $\lambda_{max} = 620$ nm, which corresponds to $\{N_2,O_x\}$ chromophore [75]. This type of coordination is confirmed by EPR results ($g_{\|} = 2.251$, $A_{\|} = 186$) (table 4). A comparison of log $K_e = 10.42$ for $Cu(Asp)H_2$(3,3,3-*tet*) (table 3) with log $K_e = 8.39$ for CuH(3,3-tri) (chromophore $\{N_2\}$) [39] indicates involvement of two nitrogens from 3,3,3-*tet* in coordination and the ligand–ligand interactions that further stabilize the complex, as observed for $Cu(Asp)H_3$(3,3,3-*tet*) species. In the ^{13}C NMR spectrum of $Cu(Asp)H_2$(3,3,3-*tet*), the shifts in the signals assigned to C(1), C(3), and C(4) close to the donor nitrogens from the amine groups of 3,3,3-*tet* are 0.074, 0.101, and 0.075 ppm, while shifts in the signals assigned to $C_{(1)}$, $C_{(4)}$, and $C_{(2)}$ Asp (relative to their positions in the spectrum of the free ligand) are 0.093, 0.125, and 0.100 ppm, which confirms the proposed mode of coordination. Analysis of the equilibrium and spectral data

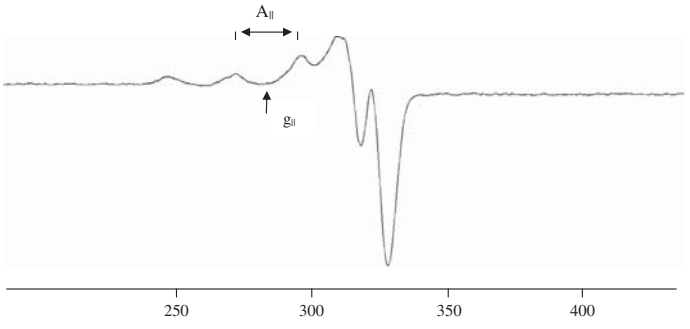

Figure 4. EPR spectra of Cu(Asp)(3,3,3-*tet*)(OH), pH 9.8.

indicates that in Cu(Asp)H_2(3,3,3-*tet*), the coordination is realized through the deprotonated terminal amine of tetramine and oxygen from the carboxyl groups of the amino acid, while the deprotonated amine from Asp and protonated amine group $-NH_2^+$ from 3,3,3-*tet* take part in the weak interactions that further stabilize the complex.

At higher pH, that is, 6.0–10, the dominant species is Cu(Asp)(3,3,3-*tet*) (log $K_e = 17.99$). At pH = 8, $\lambda_{max} = 595$ nm and EPR parameters are $g_{||} = 2.212$ and $A_{||} = 184$ (table 4). These values correspond to the {N5,O_x} type chromophore with one nitrogen in axial position as established for binary and ternary systems with PAs (a red shift is observed) [65,73]. The coordination sites were identified on the basis of ^{13}C NMR spectra of this species (table 5). At pH 8, shifts in the signals assigned to $C_{(1)}$, $C_{(4)}$, and $C_{(2)}$ from the Asp are 0.521, 0.748, and 0.118 ppm, respectively, while those of the signals of C(1), C(3), and C(4) from tetramine are 1.293, 0.357, and 1.442 ppm, respectively. These values indicate that all nitrogens from the deprotonated 3,3,3-*tet* and nitrogen from the amine group as well as oxygens from the carboxyl group of Asp are involved in the metallation.

Above pH 8, the hydroxo complexes Cu(Asp)(3,3,3-*tet*)(OH) and Cu(Asp)(3,3,3-*tet*)$(OH)_2$ are formed. Positions of the d–d bands and EPR parameters at pH 9.8 [figure 4] and pH 10.5 (table 4), at which they are dominant, respectively, point to {N4,O_x} type chromophore (the attachment of hydroxyl groups is accompanied by a shift of the absorption bands towards higher energies), which is confirmed by ^{13}C NMR results (table 5).

3.2.1. Cu(II)–Asp–*Spm* system. In the ternary system Cu(II)–Asp–*Spm*, formation of protonated complexes was detected, Cu(Asp)H_x(*Spm*) (where $x = 1$–4) and Cu(Asp)(*Spm*)(OH). The distribution of species is given in figure 2(b). At pH 4.5, at which Cu(Asp)H_4(*Spm*) is dominant, the energy of d–d transitions $\lambda_{max} = 743$ nm (table 4) points to formation of {N,O_x} type chromophore. Moreover, the composition of the species and the pH range of its occurrence suggest that it is a molecular complex of the ML$\cdots$L′ type, similar to Cu(Asp)H_4(3,3,3-*tet*). Analysis of ^{13}C NMR spectra permitted identification of the centers of interaction in this species (table 5). The metallation was found to involve oxygens from both carboxyl groups and the nitrogen from $-NH_2$ of the Asp, while the fully protonated *Spm* is in the outer sphere of coordination and takes part in weak noncovalent interactions with the anchoring Cu(Asp). This model of interaction is confirmed by analysis of changes in the d-d bands, as discussed above for Cu(Asp)H_4(3,3,3-*tet*). At pH 4.5, addition of excess *Spm* to Cu(II)–Asp does not change the position of maximum absorption [figure 3(c)]. However, introduction of an excess of Asp into the binary system

of Cu(II)–*Spm* [figure 3(d)] shifts the d–d band to shorter wavelengths, confirming coordination of copper(II) by the donor groups from Asp, while *Spm* is in the outer coordination sphere, not involved in metallation, thus the ML···L′ type complex is formed.

With increasing pH, the Cu(Asp)H_3(*Spm*) begins to form. At pH 6, the Vis and EPR results suggest formation of {N_2,O_x} type chromophore [44]. A comparison of the equilibrium constant of formation of Cu(Asp)H_3(*Spm*) log $K_e = 6.25$ with log $K_e = 5.64$ for Cu(Asp)H_2(Spd) formation [59] in which only one nitrogen of PA is engaged in the metallation, points to the involvement of –NH_2 from *Spm* in coordination and to the presence of ligand–ligand interaction further stabilizing the complex. In the ^{13}C NMR spectra, signals assigned to C(1), C(3), and C(4) from *Spm* are shifted by 0.440, 0.089, and 0.241 ppm, while those assigned to $C_{(1)}$, $C_{(4)}$, and $C_{(2)}$ from Asp are shifted by 0.442, 0.765, and 0.746 ppm, which confirms involvement of oxygens from carboxyl and –NH_2 of Asp, as well as nitrogen from the deprotonated amine of *Spm*.

At pH 6.5–9, the dominant species is Cu(Asp)H_2(*Spm*). The equilibrium constant of formation of this species log $K_e = 7.96$ (table 3) is higher than that of formation of CuH_2(*Spm*), log $K_e = 6.35$ (two nitrogens are involved in metallation) [65], which points to an additional stabilization in Cu(Asp)H_2(*Spm*), similar to Cu(Asp)H_2(3,3,3-*tet*). The position of d–d bands and EPR parameters at pH 7.8 (table 4) correspond to formation of the {N_3,O_x} type chromophore [75]. The ^{13}C NMR spectrum reveals shifts in the signals assigned to $C_{(1)}$, $C_{(4)}$, and $C_{(2)}$ from the amino acid by 0.246, 0.237, and 0.230 ppm and those assigned to C(1), C(3), and C(4) from *Spm* by 0.110, 0.030, and 0.025 ppm, which proves involvement of terminal nitrogens of PA as well as nitrogen and oxygen from Asp in coordination.

After another proton is dissociated from *Spm*, Cu(Asp)H(*Spm*) appears. In the pH range where it dominates, the UV and EPR parameters (table 4) suggest formation of {N3,O_x} type chromophore, similar to Cu(Asp)H_2(*Spm*). As follows from analysis of ^{13}C NMR spectra, the coordination centers in this species are oxygens from carboxyl and nitrogen from amine of Asp (table 5). The shifts in the signals assigned to carbons of the symmetric tetramine do not permit a reliable conclusion that nitrogens from *Spm* are directly involved in coordination. Moreover, log K_e (table 3) of this species is lower than that of the Cu(Spd) formation log $K_e = 11.70$ ({N3} chromophore [65]), which confirms the involvement of only two nitrogens from PA in coordination.

After deprotonation of the last amine from *Spm*, formation of Cu(Asp)(*Spm*) is detected. The equilibrium constant of this species is log $K_e = 11.40$, similar to log $K_e = 11.70$ for Cu(Spd) [65], in which three nitrogen donors from PA are involved in coordination. This suggests that in Cu(Asp)(*Spm*) also three nitrogens from *Spm* are involved in coordination. Unfortunately, Cu(Asp)(*Spm*) occurs in the pH range in which Cu(Asp)H(*Spm*) and Cu(Asp)(*Spm*)(OH) are present in similar concentrations, which did not permit reliable spectra of this species to identify the coordination sites.

The hydroxo complex Cu(Asp)(*Spm*)(OH) dominates at pH above 10. The spectral study (table 4) indicates formation of the chromophore {N4,O_x} [75]. Analysis of ^{13}C NMR spectra of this species at pH = 10.5 (table 5) points to involvement of oxygens from both carboxyl groups and –NH_2 of Asp in coordination. Moreover, the shifts in the signals noted for carbons prove their engagement in coordination of this ligand. Unfortunately, because of the symmetry of the *Spm* structure, it is impossible to pinpoint which nitrogens coordinate (one amine group from PA is not directly involved in interactions with Cu(II) ions).

4. Conclusions

Although the mode of complexation of Cu(II) in binary systems with aspartic acid or other amino acids similar to Asp [76–78] and in ternary systems including Asp and other ligands [79–85] has been described by many authors, no information on Cu(II)/Asp/tetramine systems has been available. In the ternary systems Cu(II)–Asp–tetramine analyzed in this work, molecular complexes of the MLL$\cdots$L′ type are formed in which the fully protonated tetramine is in the outer coordination sphere and takes part in the noncovalent interactions with the anchoring binary complex Cu(Asp). At pH 4–7 in Cu(II)–Asp–3,3,3-*tet*, the amine from Asp is excluded from metallation and takes part in noncovalent interactions with the protonated amine PA, which further stabilizes the complex. In the system with *Spm*, the $-NH_2$ from Asp is involved in coordination over the entire pH range, although only three donors of *Spm* take part in coordination. In contrast to the systems of Cu(II)/Asp/Serine or Valine [80,81] for which it has been proposed that Asp coordination takes place only through oxygen of $-C_{(1)}OO^-$ and nitrogen of the amine, in the complexes MLL′ studied in this work, also the $-C_{(4)}OO^-$ of the amino acid takes part in Cu(II) coordination. Coordination modes in the complexes with biogenic *Spm* and its shorter homologue 3,3,3-*tet* are different. According to the results of equilibrium studies, 3,3,3-*tet* forms more stable complexes with Cu(II) and Asp than *Spm*, which follows from the fact that all available donors of 3,3,3-*tet* are involved in coordination.

Copper(II) ternary complexes containing aspartic acid are interesting for brain biochemistry that could help recognize the role of Asp and PAs in living organisms.

Acknowledgment

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