Inorganic Chemistry

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Revised Coordination Model and Stability Constants of Cu(II) Complexes of Tris Buffer

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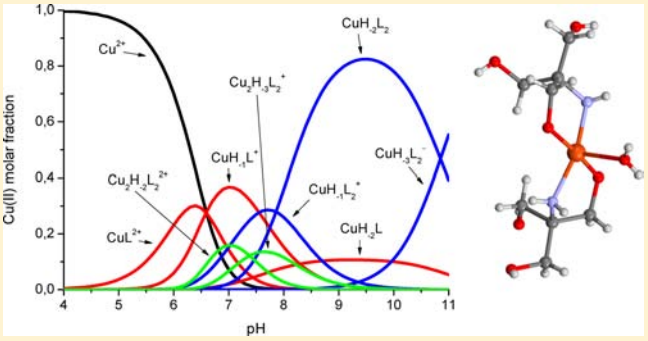
Supporting Information

ABSTRACT: 2-Amino-2-hydroxymethyl-propane-1,3-diol, or tris(hydroxymethyl)aminomethane (Tris), is probably the most common biochemical buffer used alone or in combination with other buffers because it is stable, unreactive, and compatible with most proteins and other biomolecules. Being nontoxic, it has even found applications in medicine. Tris is known, however, to coordinate transition metal ions, Cu(II) among them. Although often ignored, this feature affects interactions of Cu(II) ions with biomolecules, as Tris is usually used in high molar excess. Therefore, it is important to have precise knowledge on the stoichiometry, stability, and reactivity of cupric Tris complexes. The literature data are incoherent in this respect. We reinvestigated the complex formation in the Tris−Cu(II) system by potentiometry, UV−vis, ESI-MS, and EPR at a broad range of concentrations and ratios. We found, contrary to several previous papers, that the maximum stoichiometry of Tris to Cu(II) is 2 and at neutral pH, dimeric complexes are formed. The apparent affinity of Tris buffer for Cu(II), determined by the competitivity index (CI) approach [Krężel, A.; Wójcik, J.; Maciejczyk, M.; Bal, W. *Chem. Commun.* 2003, *6*, 704−705] at pH 7.4 varies between 2×10^6 and 4×10^4 M^{-1}, depending on the Tris and Cu(II) concentrations and molar ratio.



INTRODUCTION

2-Amino-2-hydroxymethyl-propane-1,3-diol, or tris-(hydroxymethyl)aminomethane, in brief Tris, is probably the most common biochemical buffer used alone or in combination with other buffers, being stable, unreactive, and compatible with most proteins and other biomolecules.[1] Being nontoxic, it has even found applications in human and veterinary medicine for the treatment of acidosis and in other applications.[2−4] Tris is also very frequently used in studies involving biological metal ions because it interacts very weakly with such common divalent cations, as Mg^{2+}, Ca^{2+}, or Mn^{2+}.[5−7] Unfortunately, this fact is improperly extended over transition metal ions, e.g., in tutorials and manuals.[8] On the contrary, Tris is an obvious transition metal ion chelator, as primary amine, possessing three symmetrically positioned alcoholic groups, arranged so that they can form five-membered chelate rings (Figure 1). This general property has been recognized quite a while ago, and many papers were devoted in last five decades to quantitative and qualitative descriptions of complexation of various metal ions by Tris, e.g., Ni(II), Zn(II), Ag(I), Co(II), and Cd(II).[5,7,9−12] Among these, Cu(II) seems to be the most controversial. No consensus has been achieved so far regarding the overall stoichiometry of complexes and their stabilities.[5,9,10,13−19]

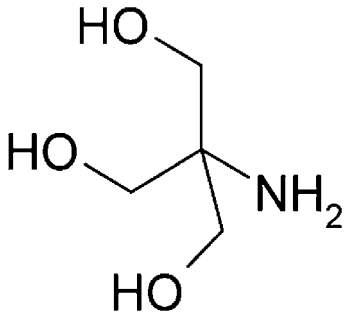


Figure 1. Structure of the Tris molecule in its deprotonated form L.

The importance of precise quantitative knowledge of Cu(II)−Tris complexes is highlighted by studies in which Tris was used specifically as a Cu(II) competitor. These studies include competition for Cu(II) binding between Tris and protein binding sites,[20] usage of Tris as an agent removing the excess of Cu(II),[21] and the application of Tris as competitor in determination of Cu(II) binding to biomolecules, e.g., PrP or Aβ peptide.[22−25] Unwanted redox activity, encountered

Received: June 7, 2013
Published: December 4, 2013

Table 1. Stability Constants of Cu(II)−Tris Complexes Determined by Potentiometry at 25 °C and I = 0.1 (KNO_3), Compared with the Literature (25 °C and I = 0.1, unless stated otherwise)[a]

ref	HL	CuL	$CuH_{-1}L$	$CuH_{-2}L$	CuL_2	$CuH_{-1}L_2$	$CuH_{-2}L_2$	$CuH_{-3}L_2$	CuL_3	CuL_4	$Cu_2H_{-2}L_2$	$Cu_2H_{-3}L_2$
5	8.13	4.05										
9	8.09	3.95	−2.05		7.63	1.31	−6.59		11.10	14.10	−1.90	
10[b]	8.11	4.17	−2.39			1.28	−6.21		11.01		−1.96	−9.01
13[c]		3.98			7.47	*	*		10.7	~13.4		
14[e]	–	4.24			7.99				11.35	13.68		
15	–								9.55			
16[d]	–	5.76			10.41							
18[c]	8.16	3.82	−2.14			1.28	−6.09					
this work	8.19(1)	4.37(2)	−2.22(1)	−10.51(2)		1.47(2)	−6.39(1)	−17.24(2)			−1.67(4)	−9.05(3)

[a]Deviations on the last digit provided by HYPERQUAD are given in parentheses.[29]. [b]I = 0.15. [c]Complexes marked with an asterisk are mentioned in the paper, but their stability constants are not provided. [d]I = 0.5. [e]I = 1.0, T = 20 °C.

sometimes in Tris buffers, has also been assigned to trace impurities of redox capable metal ions, including Cu(II).[26] Another underestimated problem is the ability of Tris to form ternary complexes. This property of Tris was demonstrated for complexes of ATP with many metal ions[5] and for the secondary Cu(II) binding site in human serum albumin.[27] Such interactions are more than likely to be a general feature of Tris coordination chemistry, as indicated recently,[19] yet neglected by other researchers, leading to significantly biased results, some of which will be discussed below.

Previously published stability constants for Cu(II) complexes of Tris were obtained by potentiometry and/or UV−vis spectroscopy. Wide discrepancies among the published results suggest that this approach was not sufficient to obtain a reliable picture. Therefore, we decided to repeat this work, using potentiometry and two spectroscopic methods suited for Cu(II) complexes, UV−vis and EPR, over a broad range of concentrations and Tris:Cu(II) ratios. As a result, we obtained a consistent set of data, which should allow for reliable application of Tris in quantitative studies of Cu(II) interactions with biomolecules.

EXPERIMENTAL SECTION

Materials. Tris, $CuCl_2$, NaOH, HNO_3, and KNO_3 were obtained from Sigma-Aldrich Co. Ethylene glycol were purchased from Merck KGaA. The pH-meter calibration buffers were received from Mettler-Toledo GmbH, Germany.

Potentiometry. Potentiometric titrations were performed on a MOLSPIN automatic titrator, using InLab 422 combined glass−Ag/AgCl electrodes (Mettler-Toledo), which was calibrated daily by nitric acid titrations.[28] The 0.1 M NaOH (carbon dioxide free) was used as a titrant. Sample volumes of 1.2−1.5 mL were used. The samples contained 1−5 mM Tris, dissolved in 6 mM HNO_3/94 mM KNO_3. The Cu(II) complex formation was studied using 1:1, 2.5:1, and 5:1 molar ratios of Tris over Cu(II), added as $CuCl_2$. All experiments were performed under argon at 25 °C, in the pH range from 2.3 to 12.2. The collected data were analyzed using the HYPERQUAD program.[29] Three titrations were included simultaneously into calculations for protonation and five for Cu(II) complexation.

UV−Visible Spectroscopy. The UV−vis spectra were recorded at 25 °C on a Cary 50 Biospectrophotometer (Varian Inc., Palo Alto, CA), over the spectral range of 240−900 nm. The optical path for all experiments was 1 cm. The samples containing Tris and Cu(II) ions were titrated with NaOH in the pH range of 3.0−11.0 by careful manual additions of very small amounts of the concentrated base solution. Changes in pH were monitored with a glass−Ag/AgCl electrode (Mettler-Toledo GmbH, Germany) calibrated directly before measurement with buffers. Cu(II) concentration was 1.0 and 10.0 mM, and the Tris:Cu(II) molar ratios were 1:1, 2.5:1, 5:1, and 100:1.

EPR Spectroscopy. The EPR spectra were recorded on a Bruker ESP 300E spectrometer in the X-band frequency (9.3 GHz) at room temperature and at 77 K. An ethylene glycol:water mixture (1:3) was used as solvent in low-temperature experiments to obtain homogeneity of the samples. The concentration of Cu(II) in the samples was 1.0 and 10 mM, and the Tris:Cu(II) molar ratios were 1:1, 2.5:1, 5:1, and 100:1. The measurements were performed in the pH range of 3.0−11.0. Additionally, serial dilution experiments were carried out for Tris:Cu(II) molar ratios 2.5:1 and 5:1 at pH values of 6.5 and 7.0, respectively, in the range of Cu(II) concentrations from 100 to 10 mM. The spectra were analyzed using WinEPR software.[30]

Electrospray Ionization Mass Spectrometry (ESI-MS). The ESI-MS spectra were recorded on a Waters Q-Tof Premier mass spectrometer equipped with the electrospray ionization source (ESI). The concentrations of Cu(II) and Tris in the samples were 10 and 25 mM, respectively. The measurements were performed at the following pH values: 5.2, 6.2, 7.0, 7.8, 8.9, and 10.3. The desired pH values were obtained by the addition of concentrated HCl or NaOH solutions. Both positive and negative ion modes were used, but only the former gave meaningful results.

RESULTS

Previous studies proposed many different complex species for the Cu(II)−Tris system, with the maximum stoichiometry varying between two and four.[5,9,10,13−16,18] In order to cover the whole range of these postulated stoichiometries, we used various Tris:Cu(II) ratios: 1.1, 2.5, and 5 in our potentiometric titrations and repeated them several times for each ratio. Several different models provided acceptable fit to this set of titrations. These models differed largely by the presence or absence of the dimeric species, which were proposed previously, but solely on the basis of potentiometric results.[9,10] Therefore, we had to resort to UV−vis and EPR spectroscopies to select the correct model. Parallel UV−vis and EPR titrations were done at two Cu(II) concentrations, 1 and 10 mM, and at the Tris:Cu(II) ratios similar those used in potentiometry. Additional spectroscopic experiments were done for the 100-fold excess of Tris over Cu(II). The final stability constants are presented in the bottom line of Table 1, which also includes all the corresponding data we were able to find in the literature. A species distribution curve for typical potentiometric experimental conditions is shown in Figure 2. Figures 3−5 show examples of UV−vis, frozen solution, and room temperature EPR spectra recorded in the pH range covering the range of complex formation indicated by potentiometry. The UV−vis spectra were similar to those presented in previous studies, with the characteristic splitting of the d−d envelope at high pH for

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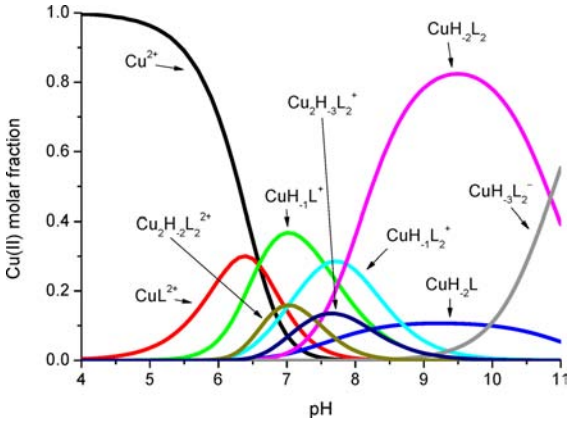

Figure 2. Species distribution of complexes calculated for 2.5 mM Tris and 1 mM Cu(II) on the basis of stability constants provided in Table 1, recorded at 25 °C and I = 0.1 (KNO_3).

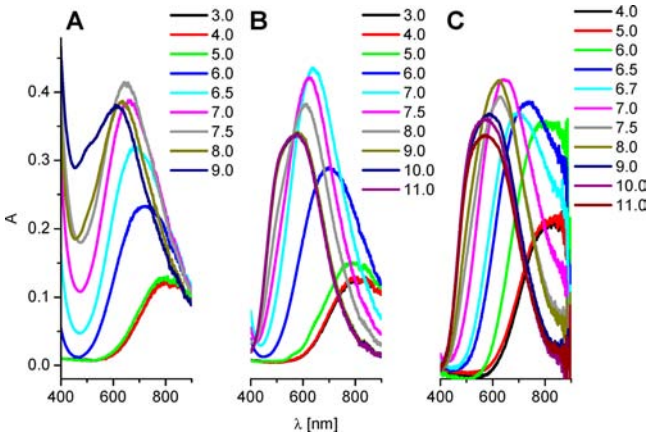

Figure 3. UV−vis pH titrations of 10 mM Cu(II) in the presence of various Tris concentrations: 11 mM (A), 25 mM (B), and 100 mM (C). Individual pH values are indicated by the respective plots. Note that the spectra at pH 3 in panels A and B overlap with those at pH 4. Likewise, the spectra at pH 10 and 11 overlap in panel B. The spectra could only be recorede up to pH 9 at 11 mM Tris (panel A) due to $Cu(OH)_2$ precipitation.

Tris:Cu(II) ratios higher than 2.[9,17−19] Precipitation could be seen in the samples with the Tris:Cu(II) ratio of 1.1 above pH 7. This was due to the formation of Cu(II) hydroxide as a result of the formation of bis-complexes.[18,19] To check if the chloride counterions would influence the coordination eqiulibria, a parallel UV−vis titration for 10 mM Cu(II) and 25 mM Tris was done, with $Cu(NO_3)_2$ instead of $CuCl_2$ as a copper source. The differences were not significant (Figure S1, Supporting Information). The comparison of species distributions calculated for spectroscopic conditions with UV−vis and frozen solution EPR spectra allowed for the assignment of spectral parameters to individual species. These are provided in Table 2. The missing UV−vis parameters for the $CuH_{-2}L$ complex could not be established reliably, due to the concomitant precipitation of traces of $Cu(OH)_2$ in UV−vis spectroscopic experiments. The better solubility of $Cu(OH)_2$ in the presence of glycol enabled the determination of EPR parameters for this species.

Two important observations helped us select the correct model of coordination. We observed that the spectral parameters recorded at the Tris:Cu(II) ratios of 2.5, 5, and

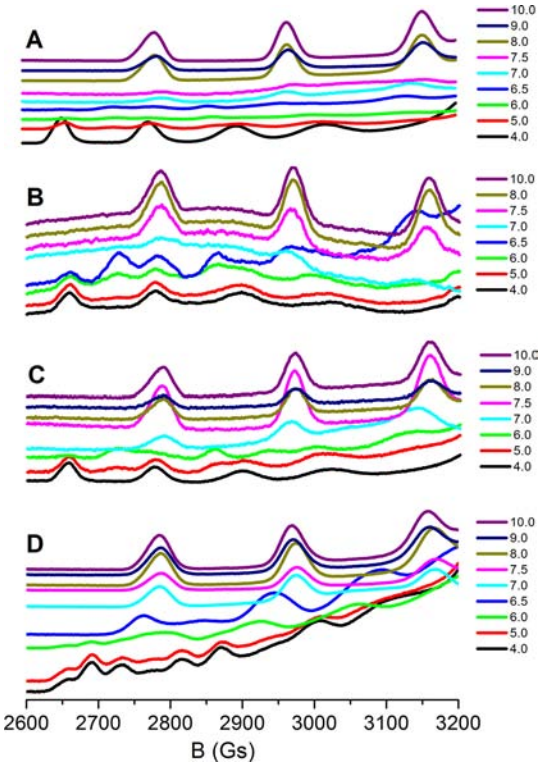

Figure 4. Parallel parts of X-band frozen solution EPR spectra recorded for 10 mM Cu(II) and various Tris:Cu(II) ratios: (A) 1.1, (B) 2.5, (C) 5, and (D) 100. Apparent pH values are indicated by the respective plots.

100 were very similar to each other. In particular, the molar ratios higher than 2.5 did not result in a significant blueshift of the *d*−*d* maximum, which would be expected if complexes with higher coordination numbers, 3 and 4, were formed. This finding was in a full agreement with the recently published thorough discussion of the previous literature.[19] The second observation was the apparent decrease in intensity of frozen solution EPR spectra around pH 6−7 (Figure 4). These spectra are presented in the first derivative form, and such an apparent decrease may be caused by several effects, such as increased microwave absorption in the frozen sample or the simultaneous presence of several species with similar parameters. On the other hand, the decrease could be caused by the formation of diamagnetic Cu(II) dimer. Therefore, we recorded room temperature EPR spectra (Figure S2, Supporting Information). These isotropic spectra, although generally less informative about the complex structure, can be easily double integrated, thus providing a rough measure of the amount of the paramagnetic species in solution. Also, the pH values recorded for them correspond to those measured in potentiometry and UV−vis samples (the addition of 30% ethylene glycol, necessary for the homogeneity of frozen solution samples affects the pH scale).[31] Indeed, the intensities of room temperature EPR spectra were also lower in the pH range of 6−7. This finding prompted us to include the dimeric complexes into the potentiometric model. Figure 5 compares the integrals of these EPR spectra with species distributions at our three typical Tris:Cu(II) molar ratios. The match of the decrease in paramagneticity of the sample with the presence of dimers is very good, thus confirming the assumed model. The EPR spectra were also reviewed for the signal at $g \sim 4$, which

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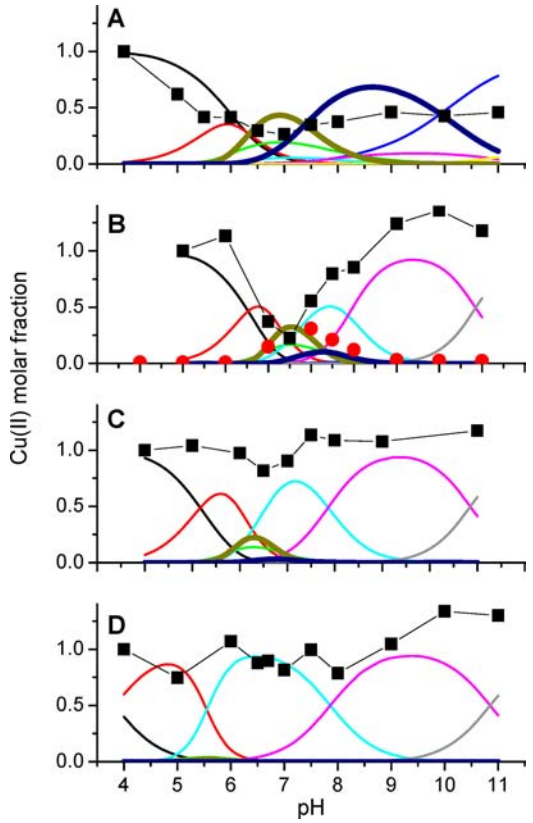

Figure 5. Comparison of intensities of the integrated room temperature EPR spectra (squares) with the potentiometry-derived species distribution for 10 mM Cu(II) and various Tris:Cu(II) molar ratios (lines): 1.1 (A), 2.5 (B), 5 (C), and 100 (D). Integrals of spectra were normalized to that recorded at pH 4 for each series. Species are color-coded in the same way as in Figure 2. Distributions of dimeric species are shown with bold lines. For a comparison, the absorption at 360 nm is overlaid for the Tris:Cu(II) ratio of 2.5 (panel B).

Table 2. Spectroscopic Parameters of Cu(II)−Tris Complexes, Obtained at 25 °C

	EPR		UV−vis	
species	$A_{\parallel}$ ($cm^{-1} \times 10^4$)	$g_{\parallel}$	λ (nm)	ε ($M^{-1}cm^{-1}$)
CuL	140	2.34	758	24
$CuH_{-1}L$	173	2.25	650	40
$CuH_{-1}L_2$	177	2.25	620	43
$CuH_{-2}L_2$ + $CuH_{-3}L_2$	184	2.24	578	34
			515[a]	32
$CuH_{-2}L$	187	2.24	[b]	[b]
$Cu_2H_{-2}L_2$ + $Cu_2H_{-3}L_2$	[c]	[c]	610	38

[a]Shoulder. [b]Parameters could not be determined due to simultaneous $Cu(OH)_2$ precipitation. [c]Diamagnetic species

would indicate the presence of the antiferromagnetic coupling between the Cu(II) ions in a dimer or a higher order oligomer. Such a signal was not detected.

Dilution EPR experiments were performed for samples where the dimeric complexes could be expected. We assumed that weak dimers might decompose upon dilution into monomeric species. This would result in the relative increase in EPR spectrum integral. We did not observe a significant effect. Calculations made on the basis of stability constants obtained for these complexes by potentiometry confirmed that the effect of dilution was not sufficiently significant, e.g., the 10-fold dilution of the solution containing 100 mM Cu(II) and 250 mM Tris, pH 6.5, which was the range of such an experiment, should result in the undetectable decrease in the population of dimers from 42% of total Cu(II) to 37%.

Independent evidence for the formation of dimeric complexes came from the UV−vis spectra. When we traced the pH dependence at the 300−400 nm region in the spectra recorded for the 2.5-fold Tris excess over Cu(II), a clear increase in the absorption in this wavelength range was noticed, as shown in Figure 5B. This coincides with previous reports correlating an absorption band in this spectral region with the presence of oxygen-bridged Cu(II) dimers, including those containing the alkoxy bridges.[32−35]

ESI-MS data were obtained for solutions of 25 mM Tris and 10 mM $CuCl_2$ at different pH values in a positive ion mode. The identified complex species are listed in Table 3. These are CuL, $CuH_{-1}L_2$, $Cu_2H_{-2}L_2$, and $CuH_{-2}L_2$ with or without Na^+ or Cl^- ion adducts, all as +1 cations. The range of existence of these complexes overlaps very well with that indicated by potentiometry (Figures 2 and 5). In particular, the doubly charged dimeric complex $Cu_2H_{-2}L_2^{2+}$ was found as a monocharged chloride adduct at pH 6.2 and 7.0, the pH range of its maximum concentration. The species predicted to exist by other models (CuL_2, CuL_3, and CuL_4 with or without Cl^- ions) were not found in the ESI-MS spectra.

DISCUSSION

Potentiometry is the method of choice for determination of stability constants of complexes of small molecules due to its high accuracy and the general character of the β constants obtained, as we discussed recently.[36] It has, however, a major weakness. The metal ion binding is observed indirectly, as perturbation of the protonation pattern of the ligand. This makes potentiometry prone to systematic errors resulting from the wrong choice of the coordination model, that is the set of stoichiometric species assumed to exist in solution in the broad pH range. The magnitude of this problem in the Cu(II)−Tris system is demonstrated in Table 1. None of previous seven studies proposed the same model, although the values of constants for identical species were often similar to each other. Also, our proposal is different from the preceding ones but has several important features in its favor. First, the maximum stoichiometry of Tris to Cu(II) is two. This was clearly demonstrated by Benn et al.[19] on the basis of UV−vis spectroscopy and before them in a rarely cited paper on β-amino alcohols by Canepari et al.[18] This fact can be also seen clearly in our potentiometric and spectroscopic data. The coordination species manifesting themselves in UV−vis and EPR spectra obtained for the Tris:Cu molar ratios of 2.5, 5, and even 100 are the same (Figures 3 and 4). Only their proportions at given pH values are different, with higher Tris excess obviously favoring the bis-complexes. Also, the significant presence of complexes in which one Cu(II) would bind more than two Tris molecules, such as CuL_3 and CuL_4, would result in the precipitation of unbound Cu(II) as hydroxide in all samples with the Tris:Cu(II) ratio of 2.5. This was not the case, but such precipitation was evident for the 1.1 ratio (Figure 3A), where the light scattering elevated the absorption at shorter wavelengths above pH 6.5. At this ratio, the formation of complexes containing two Tris molecules bound to a single Cu(II) ion (Tables 1 and 2) leaves unbound Cu^{2+} ions that precipitate as $Cu(OH)_2$ as soon as enough OH^-

Table 3. ESI-MS Data for Solutions of 25 mM Tris and 10 mM $CuCl_2$, Collected at Different pH Values in a Positive Ion Mode[a]

		pH					
complex	*m/z*	5.2	6.2	7.0	7.8	8.9	10.3
CuL^{2+}	92.00						
$CuLCl^{+}$	218.97	+	+				
$CuH_{-1}L^{+}$	183.00						
$CuH_{-1}LCl$					*Not charged*		
$CuH_{-2}L$					*Not charged*		
$CuH_{-1}L_2^{+}$	304.07		+	+	+	+	
$CuH_{-2}L_2$					*Not charged*		
$CuH_{-2}L_2Na^{+}$	326.05				+	+	+
$Cu_2H_{-2}L_2^{2+}$	183.00						
$Cu_2H_{-2}L_2Cl^{+}$	400.96		+	+			
$Cu_2H_{-3}L_2^{+}$	364.98						
CuL_2^{2+}	*152.54*						
CuL_2Cl^{+}	*340.05*						
CuL_3^{2+}	*213.08*						
CuL_3Cl^{+}	*461.12*						
CuL_4^{2+}	*273.61*						
CuL_4Cl^{+}	*582.19*						

[a]The identified complex species are CuL, $CuH_{-1}L_2$, $Cu_2H_{-2}L_2$, and $CuH_{-2}L_2$ with or without Na^+ or Cl^- ion adducts. The species predicted by other models (CuL_2, CuL_3, and CuL_4) were not found. The presence of a species at a given pH is marked by a "+". The species of our model are bold, and those proposed elsewhere but not supported by our data are italic.

ions become available. Second, the dimeric complexes, proposed previously by some authors on the basis of potentiometry alone,[9,10] were confirmed by the integration of EPR spectra, with further support from UV−vis and ESI-MS data. Figure 5 shows that the decrease in the value of the integral, resulting from the diminished presence of paramagnetic monomeric Cu(II) complexes, correlated well with the speciation of dimers, calculated from the independent potentiometric data. Figure 5B also shows the pH dependence of the UV absorption at 360 nm, which is a hallmark of oxygen-bridged dimers.[32−35] The ESI-MS experiments showed the presence of the major dimeric species, $CuH_{-2}L_2$, as a monochloride adduct at pH 6.2 and 7.0 (Table 3). Third, using various Tris:Cu(II) molar ratios, we obtained independent confirmation in frozen solution EPR spectra for the presence of all monomeric complexes indicated by potentiometry (Table 2). Fourth, the presence of major complexes, including one of the dimers, was demonstrated by ESI-MS experiments (Table 3). Fifth, the Cu(II) binding by deprotonated Tris alcoholic oxygens in all complexes formed at neutral and alkaline pH (in fact, all complexes except of CuL) is very well supported by X-ray structures of bis-complexes $CuH_{-1}L_2$[37−39] and $CuH_{-2}L_2$,[17,40,41] which invariably demonstrate such a coordination mode. The binding of both protonated and deprotonated alcoholic groups to Cu(II) is a common property of amino alcohols and amino sugars, whenever a 5- or 6-membered chelate ring including the amine group may be formed.[18,42−46] In amino sugar studies, we saw that if the configuration of the amine nitrogen and the neighboring alcoholic group enabled simultaneous coordination in the five-membered chelate ring (both axial or both equatorial) then stable complexes were formed, which involved the coordination of protonated or deprotonated alcoholic oxygen, depending on the pH. Otherwise, copper precipitated as $Cu(OH)_2$ at neutral pH.[42−46] This fact also confirms that the involvement of the Tris hydroxyl group, protonated and deprotonated, is necessary to form stable complexes, which are observed in our study. The involvement of Tris rather than the water-derived hydroxyl group is also evident from the ESI-MS because the *m/z* value was assignable to the adduct of the $Cu_2H_{-2}L_2$ dimer with one Cl^- ion, rather than a complex containing two bridging OH^- ions. Also, the X-ray structure of the Cu/Tris tetramer confirms the formation of the dimeric complex $Cu_2H_{-2}L_2$ because it is an alkoxy oxygen-bonded dimer of symmetrical $Cu_2H_{-2}L_2$ units, which in turn contain alkoxy-bridged Cu(II)−Cu(II) units.[38] Moreover, this structure contains axially bound Cl^- ions, in agreement with the ESI-MS result. The structure of the $Cu_2H_{-2}L_2$ and $Cu_2H_{-3}L_2$ dimers based on the tetramer structure is presented in Figure 6.

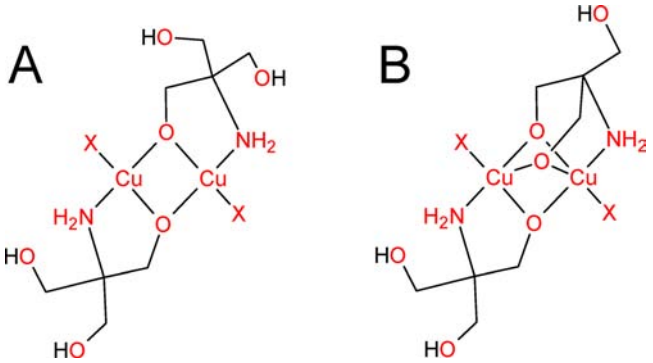

Figure 6. Probable structures of $Cu_2H_{-2}L_2$ and $Cu_2H_{-3}L_2$ dimers based on our experimental data and the published tetramer X-ray structure.[38] X represents a solvent molecule or a counterion.

In some of X-ray structures published previously, an axially coordinated water molecule was present.[17,37,39] The deprotonation of this water molecule with the p*K* value of 10.85 is very likely to be responsible for the formation of the $CuH_{-3}L_2$ complex.

The $CuH_{-2}L$ complex, detected only at a high pH (p*K* = 8.3) in solutions with the Tris:Cu(II) ratio near 1, is probably a mixed hydroxide species. Its presence was confirmed by EPR in

dx.doi.org/10.1021/ic401451s | *Inorg. Chem.* 2013, 52, 13927−13933

30% glycol (Table 2), but it is unstable in water solutions, decomposing to $Cu(OH)_2$.

Having established the coordination model for Cu(II)−Tris complexes, we can discuss its consequences for biochemical research. The first important area of its application is the use of Tris as a competitive ligand in determination of binding constants of Cu(II) complexes of biomolecules. A good example is provided by older studies of the Aβ peptide of Alzheimer's disease, where binding constant values differing by 4 orders of magnitude were obtained from similar titrations simply by using various literature models of Tris coordination.[47] It is particularly important to point out that approximation of the Tris binding properties by the stability of a *nonexistent* CuL_4 complex, or the minor CuL species, which was done in the past, e.g., in important reference materials and papers,[22,48,49] may lead to serious errors. Table 4 presents values of competitivity index (CI),[50,51] calculated for various Cu(II) and Tris concentrations, at pH 7.4. CI is the universal measure of binding ability of a given system, e.g., a set of complexes of molecule L. It is defined as log K_{MZ} (the metal M complex of a virtual competitor Z), adjusted so that it removes exactly 50% of M out of the studied system of complexes of L, $\sum_{ijk}([M_iH_jL_k]) = [MZ]$, when total concentrations of L and Z are the same. CI allows for comparisons of affinities of complexes having different stoichiometries, otherwise impossible due to different units of their binding constants. It is essentially equivalent to the calculation of free metal ion concentrations but is more feasible numerically and more intuitive, as it has a character of a surrogate conditional stability constant representing the whole system. We warn the reader that the values presented in Table 4 are only valid for given concentrations of Tris and Cu(II). The data in Table 4 show that at a high excess, which is usually the case when it is used as pH buffer, Tris binds Cu(II) with an apparent affinity of 2×10^6 M^{-1}. This binding ability is diminished by a factor of 50 when the Tris:Cu(II) ratio approaches 1 down to about 4×10^4 M^{-1}. These numbers provide a range of usage of Tris as a competitor for Cu(II) ions. It is, however, very important that explicit binding constants for all Cu(II)−Tris complex species should be used in accurate calculations. Notably, see the model of Bai and Martell,[9] presented in Table 4 for a comparison (numbers in italics give similar results to our model as long as the excess of Tris over Cu(II) is not too high). Significant deviations of 0.5−0.6 log units can be detected only at a very high Tris excess over Cu(II). This effect is not surprising because both models are derived from similar potentiometric titrations, and thus, in the presumed absence of gross experimental errors, the overall Cu(II) binding capacity of Tris from their experiments, embedded physically in titrations curves, must be similar to ours. The difference is in the deconvolution of these curves into individual components—the complex species. Thus, the two models are significantly divergent only at high Tris excess over Cu(II) and high concentrations; the conditions are very distant from those used for potentiometry. The ultimate argument in favor of our model is that we actually performed spectroscopic experiments under such conditions (Figures 3−5), and they confirmed our view.

Table 4. Competitivity Index[50,51] (CI)[a] Calculations for Various Tris and Cu(II) Molar Concentrations at pH 7.4[b]

Cu(II)/Tris	0.0001	0.001	0.01
0.001	4.67 *4.78*	4.63 *4.68*	
0.01	5.34 *5.48*	5.29 *5.42*	4.85 *4.75*
0.1	6.29 *6.92*	6.29 *6.90*	6.23 *6.77*

[a]CI is the logarithm of the conditional stability constant of MZ (the metal complex of a theoretical molecule Z), such that $\sum_{ijkl}([M_iH_jL_kA_l]) = [MZ]$, at given overall component concentrations. [b]CI is a surrogate conditional stability constant for mixtures of complex species. The CI values for the coordination model of Bai and Martell[9] (see Table 1 for details) are provided in italics.

The dimeric complexes formed at weakly acidic pH may be responsible for the reported redox side reactions of Tris buffers.[52] Our study indicates, however, that their abundance is rather low at high Tris:Cu(II) ratios. Our data allow one to design the experimental system so that such potentially deleterious complexes could be avoided.

CONCLUSIONS

Our study provided a fully documented and self-consistent view of the coordination properties of Tris buffer toward Cu(II) ions. The set of stoichiometries and binding constants presented above provides a tool for correct usage of Tris as a buffer and competitor in studies of Cu(II) binding at any binding sites of moderate affinity, including those in biomolecules.

ASSOCIATED CONTENT

Supporting Information

UV−vis pH titration of 10 mM $Cu(NO_3)_2$ with 25 mM Tris (Figure S1); room-temperature EPR pH titration of 10 mM $CuCl_2$ with 25 mM Tris (Figure S2); examples of ESI-MS spectra of 10 mM $CuCl_2$ with 25 mM Tris (Figure S3). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This study was supported by the project POIG.02.03.00-00-003/09, within the framework of Innovative Economy, the National Cohesion Strategy for Poland, co-financed by European Regional Development Fund.

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