



Cu(II) complex formation by ACES buffer



Izabela Zawisza, Małgorzata Różga, Jarosław Poznański, Wojciech Bal*

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawińskiego 5a, 02-106 Warsaw, Poland

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ABSTRACT

ACES (N-(2-Acetamido)-2-aminoethanesulfonic acid), a popular Good's buffer, binds Cu(II) ions with a moderate affinity. Although this interaction was the subject of previous studies, no consensus in the literature was found. We used potentiometry to establish binding constants, and controlled the potentiometric model selection and binding constant calculations by UV–vis spectroscopy. As a result, we obtained a consistent set of complex stoichiometries and binding constants in this system, which contains Cu^{2+} , CuL^+ , CuL_2 , $\text{CuH}_{-1}\text{L}^{-1}$ and $\text{CuH}^{-2}\text{L}^{-2}$ complexes. The negative indexes at H atoms in these formulae denote the Cu(II) assisted deprotonation of the amide nitrogen present in the ACES molecule. The affinity of ACES for Cu(II) strongly depends on the concentration and ACES:Cu(II) ratio, reaching submicromolar apparent affinities at ratios higher than 100. These results will enable more accurate determinations of biologically relevant stability constants of Cu(II) complexes using ACES buffer.

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1. Introduction

In 1960s N. E. Good with a group of his coworkers developed a series of 12 buffer agents suitable for biochemical applications [1,2]. These buffers are zwitterionic compounds based on N-substituted morpholine ring or N-substituted amino acid, either taurine or glycine. They differ by their pK_a values, in order to provide a broader range of buffer capacities, 6.15–8.35. The advantage of this new set of agents over those used earlier is that they meet most of the criteria required by biological and physiological systems including: good water solubility, low ion effect, low absorbance of light at UV–vis wave lengths, and good stability. Furthermore, the Good's buffers were designed so that they display relatively low if any ability to bind biologically relevant metal ions, including Mg(II), Ca(II), Zn(II), Cu(II), and Mn(II).

Buffer interaction with metal ions is an extremely undesired phenomenon in all experiments focusing on metal ion properties, like reactivity essays or binding experiments. In such experiments, buffer compounds are usually used at high concentrations and even weak complexing abilities may have great impact on the overall equilibrium of the studied system.

Such weak metal ion binding can be turned into advantage in determination of affinity constants for strong ligands A. If the binding constant of e.g. MA (metal–any ligand) complex lies beyond the range of constants accessible by a given method, then lowering the availability of the metal ion by the weak ligand, such as buffer B, may allow its determination through the corresponding apparent binding constant [3].

Since this approach necessitates the knowledge of stability constants of such MB (metal–any buffer) complexes, the ability of many zwitterionic buffers to form stable complexes with various metal ions has been investigated extensively.

As mentioned above, Good's buffers are not efficient ligands for the first row transition metal ions, and are often considered to be 'non-coordinating'. This notion is, however, rarely true. For example, we used potentiometry and UV–vis spectroscopy to demonstrate that Hepes buffer forms a mononuclear 1:1 Cu(II)/Hepes (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) complex with the $\log\beta_{\text{CuL}}$ of 3.22 [4]. Also other zwitterionic buffers studied appeared to be less innocent than they were thought with many of them including MES, (2-(N-morpholino)ethanesulfonic acid) MOPSO (3-morpholine-2-hydroxypropanesulfonic acid), DIPSO (N,N-bis-(2-hydroxyethyl)-3-amino-2-hydroxypropanesulfonic acid) and ACES (N-(2-acetamido)-2-aminoethanesulfonic acid, N-(carbamoylmethyl)-2-aminoethanesulfonic acid) bind divalent metal ions such as Mn(II), Co(II), Ni(II), Cu(II), Zn(II) [5–8]. Moreover, some of them were shown to form mixed ternary complexes with divalent metal ion and low molecular weight bioligands, such as nucleotides [5,7]. However, these studies were performed solely by potentiometry, which is an indirect method of research, thus prone to systematic errors.

ACES is a taurine-based Good's buffer. Its structure is presented in Fig. 1. ACES was used recently to assist calorimetric determination of the affinity of Cu(II) to Alzheimer's Disease's A β peptide and some other bioligands [3,9]. These studies, which followed a database recommendation, and assumed the sole presence of a CuL_2 ACES complex [10], prompted our interest in redetermination of Cu(II) binding to ACES. We used potentiometry to establish binding constants, but controlled the potentiometric model selection and binding constant calculations by UV–vis spectroscopy. As a result, we obtained a consistent set of

* Corresponding author. Fax: +48 22 659 4636.
E-mail address: wbal@ibb.waw.pl (W. Bal).

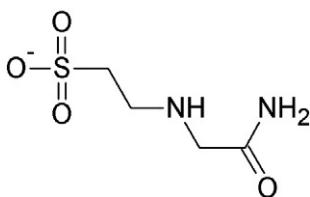


Fig. 1. Molecular structure of ACES in its deprotonated form L^- .

data which will enable more accurate determinations of biologically relevant stability constants of Cu(II) complexes using ACES buffer.

2. Experimental

2.1. Reagents

ACES buffer, Cu(II) nitrate and other reagents were purchased from Sigma-Aldrich, except of the carbonate-free 0.1 M NaOH solution, which was obtained from POCH S.A., Gliwice, Poland.

2.2. Potentiometry

Potentiometric titrations were performed on a Titrand 907 automatic titrator (Metrohm), using a combined glass-Ag/AgCl electrode (InLab® Micro, Mettler Toledo), which were calibrated daily by nitric acid titrations [11]. 0.1 M NaOH (carbon dioxide free) was used as a titrant. Sample volumes of 1.0–1.5 ml were used. The samples contained 2.5–5.0 mM ACES, dissolved in 4 mM HNO_3 /96 mM KNO_3 . The Cu(II) complex formation was studied using a double to quintuple excess of ligand over Cu(II), added as $Cu(NO_3)_2$. All experiments were performed under argon at 25 °C, in the pH range of 2.3 to 12.2. The collected data were analyzed using the SUPERQUAD and HYPERQUAD programs [12,13]. Five titrations were included simultaneously into calculations, separately for protonation and Cu(II) complexation.

2.3. UV-visible (UV-vis) spectroscopy

The UV-vis spectra were recorded at 25 °C on a Cary 50 Bio instrument (Varian Inc., Palo Alto) in the spectral range of 350–1000 nm, using 1 cm path length quartz cells. The concentrations of ACES and Cu(II) were 5 mM and 2 mM, and the molar ratios of ACES:Cu(II) used were 5, 2 and 1. The samples containing ACES and Cu(II) ions were titrated with NaOH in the pH range of 2.0–12.0, by careful manual additions of very small amounts of the concentrated base solution.

2.4. Calculations of pure spectra of Cu(II)-ACES complexes

The pH-dependent changes of Cu(II) $d-d$ spectra of ACES complexes in the 350–1000 nm region were globally analyzed with the assumption that the spectrum recorded at a given pH, $\epsilon(\lambda, \text{pH})$, is the weighted average of individual spectra of all species present in the solution, $\epsilon_i(\lambda)$ (Eq. 1). The weights, $x_i(\text{pH})$, are molar fractions of individual complexes. Concentrations of these species, $c_i(\text{pH})$ were calculated according to their β values determined by potentiometry.

$$\epsilon(\lambda, \text{pH}) = \sum_i \epsilon_i(\lambda) \cdot x_i(\text{pH})$$

$$x_i(\text{pH}) = \frac{c_i(\text{pH})}{\sum_i c_i(\text{pH})} \quad 1$$

For each wavelength (λ), the values of $\epsilon_i(\lambda)$ and accompanying standard deviations can be thus estimated with the aid of multiple regression approach against concentrations of all species, $c_i(\text{pH})$ and a set of $\epsilon(\lambda, \text{pH})$ recorded at a broad range of pH. The series of 13 spectra

Table 1

Literature values of logarithmic protonation and Cu(II) binding constants ($\log \beta$)^a of ACES, determined by potentiometry at 25 °C and $I = 0.1$ (KNO_3).

Reference	HL	CuL^+	CuL_2	$CuH_{-1}L_2^-$	$CuH_{-2}L_2^{2-}$
[1] ^b	6.9	4.6			
[6]	6.83	4.76			
[7]	7.16	5.55			
[8]	6.81	4.32	7.77	0.41	−8.08
[10]			7.77		
[15]	6.847				

^a For protonation, $\beta(\text{HL}) = [\text{HL}] / ([L^-][H^+])$; for complexes, $\beta(\text{CuH}_n\text{L}_m) = [\text{CuH}_n\text{L}_m]^{2+n-m} / ([Cu^{2+}][L^-]^m[H^+]^n)$.

^b 20 °C.

recorded in the pH range of 2.3–8.6 were then deconvoluted into five basic ones, attributed to species indicated by potentiometry, Cu^{2+} , CuL^+ , CuL_2 , $CuH_{-1}L_2^-$ and $CuH_{-2}L_2^{2-}$. Calculations were done using the Gnuplot program [14].

3. Results and discussion

Potentiometry is the method of choice for determination of protonation and complex formation constants of small molecules [15]. We used it to study ACES-Cu(II) interactions, but, in contrast to the all but one previous study of this system [8], we employed UV-vis spectroscopy to control the potentiometric calculations and derive the Cu(II) binding modes. The literature data are collected in Table 1 [1,6–8,10,16].

Preliminary UV-vis tests indicated the significant $Cu(OH)_2$ precipitation at ACES:Cu(II) molar ratios lower than 2, thus indicating the formation of bis-complexes, contrary to several previous reports, which listed only the CuL species [1,6,7]. Therefore, all further potentiometric studies were performed for ACES:Cu(II) molar ratios of 2 and higher. UV-vis experiments indicated, however, that precipitation of hydroxide was fully prevented only at the highest molar ratio used, 5. Therefore, the calculations of stability constants were made on the basis of titrations of samples containing a five-fold excess of ACES over Cu(II). The results of stability constant calculations are presented in Table 2, while Fig. 2 shows the species distribution plot, validated by the pH dependence of the $d-d$ band intensity. The corresponding UV-vis spectra are presented in Fig. 3. In order to obtain spectral parameters of individual complex species, these spectra were deconvoluted into pure components, according to the potentiometric species distribution. Fig. 4 shows these components, while their parameters are included in Table 2. The correctness of this approach is confirmed by the very low standard deviations of deconvoluted spectra, visualized in this figure.

The coordination model obtained from the fitting of potentiometric curves includes the species with negative hydrogen stoichiometries, $CuH_{-1}L_2^-$ and $CuH_{-2}L_2^{2-}$ (Fig. 2). Such species are common in Cu(II) complexes of amides and peptides, where negative indices result from the metal-assisted release of protons from amide nitrogens [17–19]. These protons do not dissociate spontaneously in the accessible pH range, and therefore are not included in the protonation pattern of the

Table 2

Logarithmic protonation and Cu(II) binding constants ($\log \beta$ ^a and pK_a) of ACES, determined by potentiometry at 25 °C and $I = 0.1$ (KNO_3), and UV-vis spectral parameters of respective complexes. Statistical errors on the last digits of $\log \beta$ values, provided by HYPERQUAD [12], are given in parentheses.

Species	$\log \beta$	pK_a	ΔK	UV-vis	
				λ_{max} (nm)	ϵ ($M^{-1} \text{cm}^{-1}$)
HL	6.757(2)				
CuL^+	4.38(1)	−2.38	770	35	
CuL_2	8.04(1)	−5.48	694	36	
$CuH_{-1}L_2^-$	1.15(1)	6.89	635	53	
$CuH_{-2}L_2^{2-}$	−7.19(1)	8.34	549	62	

^a For complexes, $\beta(\text{CuH}_n\text{L}_m) = [\text{CuH}_n\text{L}_m]^{2+n-m} / ([Cu^{2+}][L^-]^m[H^+]^n)$.

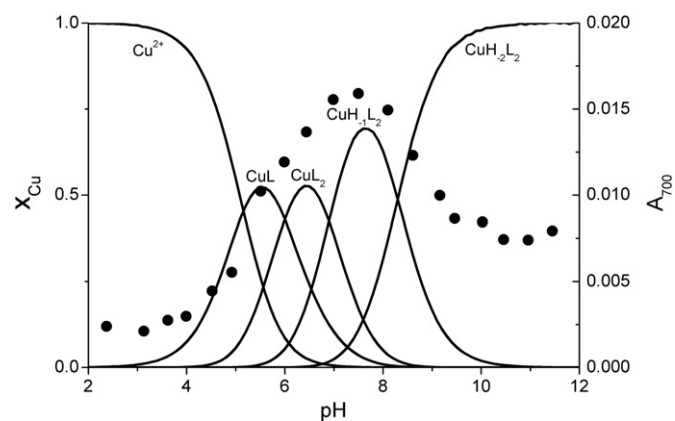


Fig. 2. Species distribution plots, calculated for 2 mM ACES and 0.4 mM Cu(II). Stoichiometries of species are indicated on the plot.

free ligand. However, in the framework of HYPERQUAD calculations, such “negative protons” are equivalent to hydroxyl groups, and stoichiometries of these two complexes might actually be $\text{CuL}_2(\text{OH})^-$ and $\text{CuL}_2(\text{OH})_2^{2-}$. In order to solve this ambiguity, we inspected the parameters of UV-vis spectra and compared them with potential complex structures, using analogies from amide and peptide studies.

ACES molecule contains three potential metal-binding groups: terminal sulfonate and amide, with the intervening secondary amine. The sulfonate is not a significant binding site from the point of view of Cu(II) binding, and thus, in the first approximation, ACES can be considered as N-substituted glycineamide. Cu(II) binding to this molecule has been studied quite extensively [20–24] and the results published before 1982 were discussed in the classical review on the coordination properties of the amide bond by Sigel and Martin [17]. Although the models proposed in these papers differ in details, most of them propose the existence of species similar to those found by us for ACES. The complexes with negative protons were assigned in those studies to the deprotonation of the amide nitrogen. The pK_a values for the first and second amide deprotonation in the CuA_2 complex of glycineamide were 6.7–7.0 and 8.1–8.2, respectively [21,23,24]. The corresponding values found by us for ACES are very similar: 6.89 and 8.34. On the other hand, the stability constants of CuL^+ and CuL_2 complexes for ACES, corrected for amine protonation (*K), (Table 2) are higher from those of glycineamide, by 0.3 and 1 log units, respectively [22–24]. In order to further confirm this assignment for ACES complexes, we resorted to the empirical formula defined by Sigel and Martin, which correlates the λ_{max} of the $d-d$ band

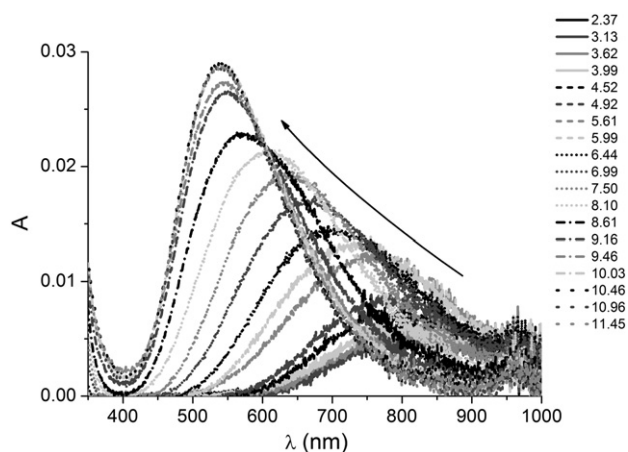


Fig. 3. UV-vis spectra at 25 °C of samples containing 2 mM ACES and 0.4 mM Cu(II), with $I = 0.1$ (KNO_3) in the pH range of 2.3 to 11.5. The arrow indicates the direction of spectral changes upon the increase of pH.

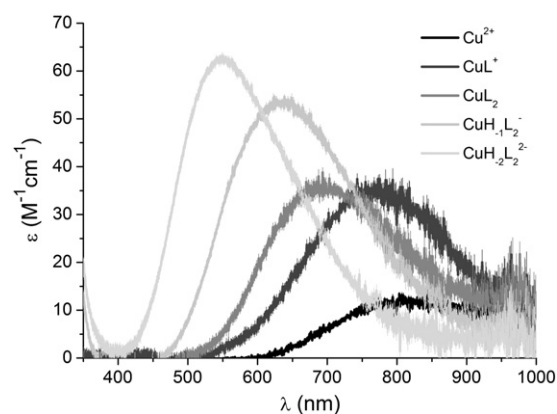


Fig. 4. Spectra of pure Cu(II) complex forms obtained from data presented in Fig. 3 by deconvolution on the basis of the potentiometric species distribution. Standard deviations for each wavelength are indicated as vertical lines.

of the Cu(II) ion with the character of coordinating ligands (Eq. 23 in Ref [17]). We assumed the following possibilities of the equatorial coordination modes: in CuL^+ and CuL_2 complexes the ACES molecule is coordinated by amine and either a carbonyl oxygen or a sulfonate (we used the carboxylate coefficient for this purpose), while in $\text{CuH}_{-1}\text{L}_2^-$ and $\text{CuH}_{-2}\text{L}_2^{2-}$ complexes the alternative is between the deprotonated amide and the water-derived hydroxyl group. The calculations are summarized in Table 3, which clearly indicates that the experimental λ_{max} values are systematically red-shifted from the calculated ones. Noting the fact that apical coordination of a negatively charged oxygen donor red-shifts the $d-d$ band by 30–50 nm [17,25], and the sulfonate is sterically capable of such binding, forming a 6-membered chelate ring, we assume that the actual structures of all complexes involve apical binding of one or two sulfonates, and the deprotonated amide nitrogen occupies the equatorial position(s) in $\text{CuH}_{-1}\text{L}_2^-$ and $\text{CuH}_{-2}\text{L}_2^{2-}$ complexes. This arrangement also accounts for the relative increase of stability of the ACES complexes, compared to glycineamide. Fig. 5 presents the postulated structures of the complexes discussed above.

We have to note that the coordination model obtained in this work is qualitatively identical with that published previously, also on the basis of potentiometry and UV-vis spectroscopy [8]. These models differ quantitatively, however, especially in the area of deprotonated species, which are more stable in our model. Another difference is that the previous model denied sulfonic group coordination and assumed square-planar structures of complexes.

The relatively high stability of the CuL_2 complex results in a non-linear dependence of the ability of ACES to sequester Cu(II). In order to facilitate the analysis we calculated the competitiveness index (CI) [26,27] calculated for various Cu(II) and ACES concentrations, at pH 7.4. CI is the universal measure of binding ability of a given system, e.g. set of

Table 3

The comparison of experimental values of λ_{max} (nm) for individual ACES complexes with those predicted from the empirical formula proposed by Sigel and Martin [16].

Species	λ_{max} (nm)			
	experimental	$\text{NH}_2 + \text{N}^-^a$	$\text{NH}_2 + \text{OH}^-^b$	$\text{NH}_2 + \text{SO}_3^-^c$
CuL^+	770	745	745	717
CuL_2	694	663	663	621
$\text{CuH}_{-1}\text{L}_2^-$	635	585	663	
$\text{CuH}_{-2}\text{L}_2^{2-}$	549	524	663	

^a N^- assumed to account for negative stoichiometric index at H in $\text{CuH}_{-1}\text{L}_2^-$ and $\text{CuH}_{-2}\text{L}_2^{2-}$.

^b OH^- assumed to account for negative stoichiometric index at H in $\text{CuH}_{-1}\text{L}_2^-$ and $\text{CuH}_{-2}\text{L}_2^{2-}$.

^c SO_3^- assumed to bind equatorially in CuL^+ and CuL_2 .

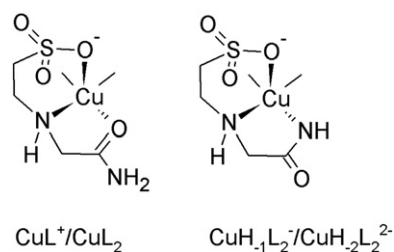


Fig. 5. ACES binding modes in Cu(II) complexes. Only one ligand is shown for clarity.

complexes of molecule L. It is defined as the $\log K_{\text{MZ}}$ (the metal M complex of a virtual competitor Z), adjusted so, that it competes exactly 50% of M out of the studied system of complexes of L, $\sum_{ijk}([M_iH_jL_k]) = [\text{MZ}]$, when total concentrations of L and Z are the same. CI facilitates the comparisons of binding abilities of complexes of different stoichiometries, otherwise rather difficult, because the respective binding constants have different units. CI is principally equivalent to the calculation of free metal ion concentrations, but is more feasible numerically and more intuitive, as it has a character of an apparent affinity constant. The bold typeface data in Table 4 show that at a high excess, which is usually the case when it is used as pH buffer, ACES binds Cu(II) with an apparent affinity of $3.3 \times 10^7 \text{ M}^{-1}$. This binding ability is diminished by a factor of 50 when the ACES:Cu(II) ratio approaches 1, down to ca. $2.2 \times 10^4 \text{ M}^{-1}$. These numbers provide a range of applications of ACES as competitor for Cu(II) ions. It is, however, very important, that explicit binding constants for all complexes should be used in accurate calculations.

The regular typeface data in Table 4 were calculated on the basis of the previously used data (Refs. [10] and [15]). The comparison of two datasets of Table 4 shows that the previously used data underestimate the binding abilities of ACES at pH 7.4, by up to one order of magnitude at a high molar excess, in terms of the apparent affinity constant expressed in M^{-1} . The contribution of the CuL_2 species to this difference is only 0.13 log units per ACES molecule (Tables 1 and 2). The bulk of the effect is provided by the $\text{CuH}_{-1}\text{L}_2^-$ complex, which is the actual major species at pH 7.4 (see Fig. 2).

4. Conclusion

We demonstrated that ACES buffer is a relatively strong Cu(II) chelator, forming bis-complexes which involve deprotonated amide nitrogen coordination. At a high molar excess over Cu(II), which is a typical situation in the laboratory practice, ACES can bind Cu(II) ions with an effective affinity higher than micromolar. The data presented above provide solid background for applications of this buffer/chelator as competitor in determinations of stabilities of Cu(II)/biomolecule studies.

Abbreviations

ACES	(N-(2-acetamido)-2-aminoethanesulfonic acid, N-(carbamoylmethyl)-2-aminoethanesulfonic acid)
CI	competitiveness index
*K	protonation corrected stability constant
L	fully deprotonated form of ACES

Table 4

The competitiveness index (CI^a [26,27]) calculations for various ACES and Cu(II) molar concentrations, at pH 7.4. Values calculated from stability constants determined in this work (bold) are compared with those obtained for previously proposed constants of $\log \beta(\text{CuL}_2) = 7.77$ [10] and $\text{p}K_a$ of HL = 6.847 [15].

Cu(II)	0.0001	0.001	0.01
ACES			
0.001	5.48 4.50	4.48 3.86	
0.01	6.52 5.55	6.45 5.49	4.35 3.49
0.1	7.52 6.56	7.51 6.55	7.45 6.49

^a CI is the logarithm of the conditional stability constant of MZ (the metal complex of a theoretical molecule Z), such that $\sum_{ijkl}([M_iH_jL_kA_l]) = [\text{MZ}]$, at given overall component concentrations. Here concentrations of ACES and Z are set equal.

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