Solution Properties of Cu(II)–Sarcosinehydroxamic Acid

BARBARA KURZAK*
Institute of Chemistry, Pedagogical University, 08-110 Siedlce, Poland
and JULIA JEZIERSKA
Institute of Chemistry, University of Wrocław, 50-383 Wrocław, Poland

(Received September 29, 1987; revised March 9, 1988)

Abstract

Species distribution and relevant stability constants of species present in aqueous solutions of Cu(II) with bidentate sarcosinehydroxamic acid ($CH_3NHCH_2CONHOH$=HL) have been obtained by analytical potentiometry at 25 °C and $I = 0.1$ M ($NaClO_4$). Together with ESR of frozen solutions measurements, these results show the existence of four different complex copper(II) species, one of which is a dimeric one. The solution electronic spectra are also reported. The structure of the complexes are discussed and conclusions are drawn based on absorption and ESR spectra.

Introduction

Hydroxamic acids have important and diverse functions in biochemical systems. Their oxidized peptide group, –CON(OH), has been found in a number of natural products such as antibiotics and bacterial growth factors [1]. The hydroxamic acids are also known to have antituberculous, antifungous and antileucemic activities [2, 3]. Brown *et al.* [4–6] have suggested some chemical criteria which are useful in the eventual aim of designing metal chelates as suitable sources of various trace elements essential in animal nutrition. According to them, the chelate must be stable, remain monomeric at biological pH values, must be able to undergo rapid iron exchange with apotransferrin and the free ligand should be able to extract iron fairly rapidly from ferritin. Ferric acetohydroxamate [4] and ferric glycinehydroxamate [7] satisfied all the criteria for biological activity giving a strong indication for these chelates as suitable sources of iron in animal anemia.

In addition to iron as a trace element, copper is another whose biological role is well known as essential for living organisms. It has been shown, that deficiency of copper in the nutrition causes different human and animal diseases, such as copper deficiency anemia and genetic defects in copper metabolism [8]. Hence, it seems to be important to estimate the coordination properties of amino hydroxamic acids to Cu(II) ions. On the other hand, the choice of Cu(II) as a metal centre coordinated by amino hydroxamic acids, allows use of the ESR method to study the donor properties of these chelate agents. ESR proved to be a valuable and unique tool for determining the nature of the close-lying donors of the Cu(II) ions [9].

The interactions of Cu(II) ions with sarcosinehydroxamic acid ($CH_3NHCH_2CONHOH$=HL) were investigated. As in previous papers [10, 11], a numerical method (SCOGS) was used in order to determine the stability constants and to choose a chemical model. Probable structures of the metal chelate compounds are suggested. Evidence is given for a dimeric Cu(II)–sarcosinehydroxamic acid complex on the basis of ESR spectra and potentiometric titration.

Experimental

Reagents and Materials

Sarcosinehydroxamic acid (HL) was prepared by mixing ice-cold methanol solutions of sarcosine methyl ester (0.1 mol) and hydroxylamine (0.1 mol). When the mixture was cooled, HL crystallized. Recrystallization from a methanol/aqueous solution of acetic acid (1%) gave pure crystals: yield 50%. *Anal.* Calc.: C, 34.61; H, 7.74; N, 26.91. Found: C, 34.90; H, 7.69; N, 26.92%.

Bidistilled water was used throughout and all titrations were carried out under an atmosphere of purified argon. All reagents were of analytical grade. Stock solution of copper(II) perchlorate (0.1 mol) was prepared by dissolving a proper amount of copper(II) perchlorate hexahydrate in water. The exact concentration of the solution was determined by the iodometric method.

*Author to whom correspondence should be addressed.

0020-1693/88/$3.50

Potentiometric Titrations

Measurements of pH were carried out on an OP-208/1 pH meter (Radelkis) with a digital readout, equipped with OP 7183 glass and OP 830 saturated calomel (containing NaCl) electrodes. The electrode system was calibrated by periodic titrations of perchloric acid (or sodium hydroxide) solution (0.1 M in $NaClO_4$) with standard sodium hydroxide (or perchloric acid) solution. The resulting titration data was used to calculate the standard electrode potential E° and the dissociation constant for water. These values were then used in the calculation of hydrogen ion concentration $[H^+]$ from potential readings [12]. pH was calculated from the equation: $pH = -\log f[H^+]$. Activity coefficient, f, of hydrogen ions was calculated from the equation given by Davis [13].

Potentiometric titrations were carried out on a series of solutions (20 ml total volume) 0.1 M in $NaClO_4$ and sufficient $HClO_4$ to bring the starting pH close to 2.5. The measuring system was thermostated at 25 ± 0.1 °C. Small amounts of base were added with the use of a micropipette.

Ligand titrations were performed in the absence of copper ions (in this case, titration curves were obtained by varying the initial concentration of sarcosinehydroxamic acid at 1.0×10^{-2} and 1.6×10^{-2} M) and in the presence of copper ions ($2.7-4.0 \times 10^{-3}$ M) (in this case, the concentration ratios of ligand to metal ions varied in the range from 2.5:1 to 6:1). Formation constants were calculated using the Bjerrum method [14] and a modified version of the computer program SCOGS [15]. Species distribution as a function of pH was determined with the COMICS computer program [16].

ESR Measurements

The ESR spectra were obtained using a JES-ME X-band spectrometer, proton magnetometer and ESR standards, at 252 and 170 K. The samples were prepared from a stock solution containing 0.1 M $NaClO_4$, 4×10^{-3} M copper(II) ions and 1×10^{-2} M sarcosinehydroxamic acid in bidistilled water. The pH was adjusted with 1.0 M NaOH solution.

Spectrophotometric Measurements

Absorption in the region 400–900 nm was obtained on a SPECORD M-40 (C. Zeiss, Jena) spectrophotometer and solutions were scanned at a series of pH values from pH 2 to 11 by using 1-cm quartz cells. The stock solution was 0.1 M in $NaClO_4$, 4×10^{-3} M in Cu(II), 1×10^{-2} M in HL and 2×10^{-2} M in $HClO_4$. The pH was adjusted by adding 1.0 M NaOH solution.

Results and Discussion

Titrations

Sarcosinehydroxamic acid (HL) is able to coordinate an additional proton to the substituted amino group (CH_3NH-), $pK = 7.62$ and contains one ionizable proton in its hydroxamic acid group ($-NHOH$), $pK_a = 9.20$. Comparison of the pK for sarcosinehydroxamic acid with that of glycinehydroxamic acid ($pK = 7.10$ [17]) shows that substitution of a $-CH_3$ group for the H of the $-NH_2$ group increases the pK.

Evaluation of β_{pqr} constants, using the computer program SCOGS, gave a four copper(II) species. The calculated concentrations of this four copper(II) species, as a function of pH, in solution containing 4×10^{-3} M Cu(II) and 1×10^{-2} M sarcosinehydroxamic acid indicate that under these conditions the CuL^+ species is not more than 14% of the total copper content in solution, while the dimeric species, at pH = 5.25, reaches 93%. The species CuL_2, from pH = 7.5 to 8.5, corresponds to over 90%, with a maximum of 98.5% at pH 7.7. The resulting formation constants are given in Table I and the species distribution is given in Fig. 1. Similar results were obtained for systems Cu(II)/glycinehydroxamic acid [18], Cu(II)/L-α-alaninehydroxamic acid [10] and Cu(II)/L-α-leucinehydroxamic acid [11] but in the investigated system all stability constants are much lower than that in those systems. The reason is not apparent at present. It is possible that steric

TABLE I. Logarithmic Stability Constants ($\log \beta_n$) of Complexes Species $M_pH_qL_r$ (M = Cu, L = sarcosinehydroxamate ion), $I = 0.1$ ($NaClO_4$), $T = 25$ °C

p	q	r	Numerical method (SCOGS)	Bjerrum method
0	1	1	9.20(1)	9.25
0	2	1	16.82(1)	16.80
1	0	1	10.39(9)	11.30
2	−1	2	20.22(3)	
1	0	2	18.52(3)	18.43
1	−1	2	8.77(4)	

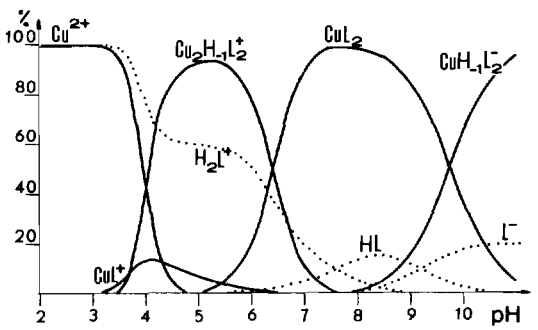

Fig. 1. Species distribution in the Cu(II)/sarcosinehydroxamic acid system as a function of pH, $c_M = 4 \times 10^{-3}$ M; $c_L = 1 \times 10^{-2}$ M. Percentage of the species refer to total metal except for the metal-free forms, which refer to total ligand.

hindrance is responsible, but we have little information about the variability of β values for Cu(II) complexes of substituted amino hydroxamic acid.

Electron Spin Resonance Spectra

The ESR spectra of Cu–sarcosinehydroxamic acid as a function of pH are in general similar to those observed recently for related systems derived from glycine [18], alanine [10] and leucine [11], but its liquid solution spectra are much better resolved providing the possibility to calculate and to analyse the g_0 and A_0 isotropic parameters.

The solution ESR spectrum at the lowest pH (2.45) shows (Fig. 2) a single, broad line characteristic for Cu(II) ions coordinated by H_2O molecules. The aquo environment of Cu(II) ions at this pH is confirmed by the frozen solution spectrum (Fig. 3). A four line hyperfine structure of the room temperature spectrum at pH = 3.30 indicates the formation of the CuL^+ complex. Its increasing contribution (in

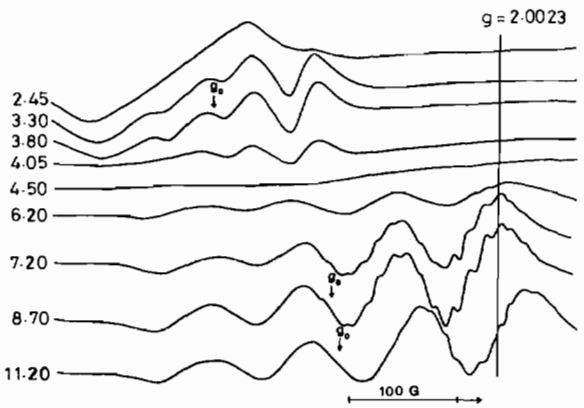

Fig. 2. ESR spectra of Cu(II)/sarcosinehydroxamic acid as a function of pH; $c_M = 4 \times 10^{-3}$, $c_L = 1 \times 10^{-2}$ M; solvent: H_2O; temperature: 252 K; microwave frequency: 9.421 GHz.

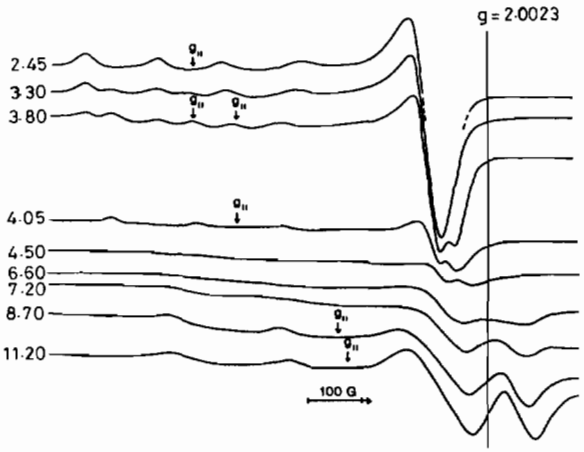

Fig. 3. ESR spectra of Cu(II)/sarcosinehydroxamic acid as a function of pH; $c_M = 4 \times 10^{-3}$ M, $c_L = 1 \times 10^{-2}$ M; solvent: H_2O; temperature: 170 K; microwave frequency: 9.127 GHz.

TABLE II. The Parameters of the ESR Spectra

	pH				
	2.45	3.80	4.50	8.70	11.2
Form	Cu(II) aquo	CuL^+	$Cu_2H_{-1}L_2^+$	CuL_2	$CuH_{-1}L_2^{-1}$
g_0	2.189	2.172		2.101	2.097
A_0 [a]		56		88	95
$g_\parallel$	2.400	2.327		2.186	2.172
$A_\parallel$ [a]	146	171		210	220

[a] $cm^{-1} \times 10^{-4}$.

equilibrium with Cu(II) aquoions) with increase in pH from 3.30 to 3.80 is demonstrated by frozen solution spectra. The relatively high value of g_0 and $g_\parallel$ and small value of A_0 (Table II) may be associated with tetrahedral distortion [19] of asymmetric coordination in the CuL^+ complex realized by two nitrogen atoms of the ligand and water molecules completing the ligation of Cu(II). At pH = 4.05 the spectra apparently decline (Fig. 2 and Fig. 3), in agreement with the decrease in the concentration of the CuL^+ complex accompanying the formation of the dimeric $Cu_2H_{-1}L_2^+$ complex. The almost complete disappearance of the spectra between pH = 4.50 and 5.80 is consistent with the predominant contribution of the dimeric complex in this pH range. As the pH increases above 6, the liquid solution spectrum reappears and the frozen solution spectrum exhibits the new structure. This corresponds to the formation of the new CuL_2 species. The improvement of the resolution of the spectra achieved at pH about 8 coincides with the maximum contribution of this complex. The decrease of g_0 and $g_\parallel$ and increase of A_0 and $A_\parallel$ values (Table II), associated with the increase of the number of equatorial ligation [9], confirms the involvement of four nitrogen atoms in the coordination to Cu(II) in the CuL_2 complex. Additional information about this complex is provided by the superhyperfine splitting (of about 14 G) observed in the room temperature spectra at pH 7.2 and 8.7, considered as the result of the interaction of Cu(II) and four ^{14}N atoms (Fig. 2). The spectra (both at room and low temperature) achieved at pH = 11.4 correspond to the prevailing contribution of the new $CuH_{-1}L_2^{-1}$ complex. Although the ESR data (Fig. 2 and Fig. 3, Table II) relevant to this complex support a similar N_4 donor set around Cu(II), the g_0 and $g_\parallel$ values are smaller and A_0 and $A_\parallel$ are higher in comparison with the CuL_2 complex. This trend is consistent [9] with the more negative total charge of the $CuH_{-1}L_2^{-1}$ complex.

Electronic Spectra/pH Profile

Formation of the different copper(II) sarcosinehydroxamate complexes with increasing pH can be followed by absorption spectroscopy in the 400–900

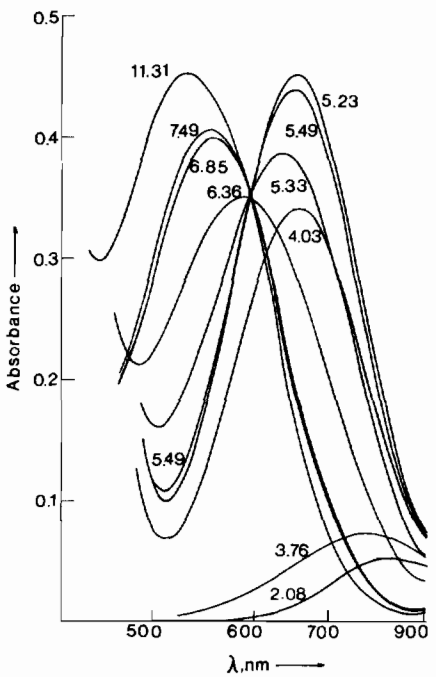

Fig. 4. Visible spectra of Cu(II)/sarcosinehydroxamic acid as a function of pH, $c_M = 4 \times 10^{-3}$ M; $c_L = 1 \times 10^{-2}$M, (1 cm cells).

nm range. The spectra in Fig. 4 were obtained under exactly the same conditions used in the calculation of the species concentrations shown in Fig. 1. The absorption spectra of Cu(II)–sarcosinehydroxamic acid exhibit, in general, approximately the same changes with increasing pH to those stated for the above mentioned systems. Some differences are observed in the energy of the characteristic absorption maxima. The formation of the complexes between Cu(II) and the investigated ligand begins at pH about 3 (Fig. 4). The maximum of the broad spectrum near the infrared region shifts towards the visible region with increase of pH. At pH 5.25 the maximum occurring at 646 nm corresponds to the greatest concentration of the dimeric complex $Cu_2H_{-1}L_2^+$ (Fig. 1). The equilibrium results [18] indicate the presence of one OH^- bridging group in this complex. Since, the complex $Cu_2H_{-1}L_2^+$ exhibits λ_{max} at 646 nm, it is possible to conclude that one copper cation is coordinated by two nitrogen atoms of the ligand [10].

The characteristic isosbestic point seen at 580 nm, is associated with an equilibrium between the species $Cu_2H_{-1}L_2^+$ and CuL_2. The maximum at 545 nm does not change its energy from pH = 7.4 to 8.4 (Table III), which corresponds to the predominant concentration of CuL_2 complex in this pH range (Fig. 1). The appearance of the absorption maximum at 545 nm suggests the coordination of one copper cation by two ligands forming two five-membered rings and

TABLE III. Spectrophotometric Data for CuL_2 Species as a Function of pH (range 6–10)

pH	Band maxima (nm)	Absorption
6.390	562	0.36
6.846	548	0.40
7.410	545	0.41
7.574	545	0.41
7.767	545	0.41
7.965	545	0.41
8.135	545	0.41
8.397	545	0.41
8.609	541	0.41
9.429	541	0.41
10.106	535	0.41

supports its CuL_2 formula [10]. On the basis of the results of Gaussian analysis of electronic spectrum [20], the diagram for CuL_2 of D_{4h} symmetry was assumed to be relevant for this species. The next shifting of the maximum with further increase in pH above 9.0 is consistent with the formation of the new $CuH_{-1}L_2^{-1}$ complex.

Conclusions

The above potentiometric, spectroscopic and ESR results show that sarcosinehydroxamic acid coordinates to Cu(II) via the α-amino nitrogen and the nitrogen atom of the –CONHOH group and that the aqueous solution contains the dimeric species in the pH range 4.5 to 5.8. In the pH range 7.4–8.4 only the CuL_2 species appears to be present. The decrease of g_0 and $g_\parallel$ and increase of A_0 and $A_\parallel$ values, associated with an increase of the number of equatorial ligations, confirms the involvement of four nitrogen atoms in the coordination to Cu(II) in the CuL_2 complex. On the basis of the results of a Gaussian analysis of the electronic spectrum the diagram for the CuL_2 species of D_{4h} symmetry was assumed to be relevant for this species. The ESR spectra achieved at pH = 11.4 correspond to the prevailing contribution of the $CuH_{-1}L_2^{-1}$ complex. For this complex the g_0 and $g_\parallel$ values are smaller and A_0 and $A_\parallel$ are higher in comparison with the CuL_2 complex. This trend is consistent with the more negative total charge of the $CuH_{-1}L_2^{-1}$ complex.

References

1 J. B. Neilands, *Struct. Bonding (Berlin), 1,* 59 (1966).
2 R. T. Coutts, *Can. J. Pharm. Sci., 2,* 27 (1967).
3 R. T. Coutts, *Can. J. Pharm. Sci., 2,* 1 (1967).
4 D. A. Brown, M. V. Chidambaram, J. J. Clarke and D. M. McAleece, *Bioinorg. Chem., 9,* 255 (1978).

5 D. A. Brown and B. S. Sekhon, *Inorg. Chim. Acta, 91,* 103 (1984).
6 D. A. Brown and A. L. Roche, *Inorg. Chem., 22,* 2199 (1983).
7 D. A. Brown, M. V. Chidambaram and J. D. Glennon, *Inorg. Chem., 19,* 3260 (1980).
8 Ciba Foundation Symposium; 79 (new series) (ed), 'Biological Roles of Copper', Excerpta Medica, Elsevier/ North Holland, Amsterdam, 1980.
9 J. Peisach and W. E. Blumberg, *Arch. Biochim. Biophys., 165,* 691 (1974).
10 B. Kurzak, K. Kurzak and J. Jezierska, *Inorg. Chim. Acta, 125,* 77 (1986).
11 B. Kurzak, K. Kurzak and J. Jezierska, *Inorg. Chim. Acta, 130,* 189 (1987).
12 M. Molina, C. Melios, J. O. Tognalli, L. C. Luchiari and M. Jafelicci, Jr., *J. Electroanal. Chem., 105,* 237 (1979).
13 C. W. Davis, *J. Chem. Soc.,* 2093 (1938).
14 J. Bjerrum, 'Metal Ammine Formation in Aqueous Solution', P. Haase, Copenhagen, 1941.
15 I. G. Sayce, *Talanta, 15,* 1397 (1968).
16 D. D. Perrin and I. G. Sayce, *Talanta, 14,* 833 (1967).
17 B. Kurzak, unpublished results.
18 E. B. Paniago and S. Carvalho, *Inorg. Chim. Acta, 92,* 253 (1984).
19 H. Yokoi and A. W. Addison, *Inorg. Chem., 16,* 1341 (1977).
20 K. Kurzak and B. Kurzak, in preparation.