

CCA-1840

YU ISSN 0011-1643

UDC 541.122

Note

Equilibrium Studies of Binary and Ternary Complexes of Cycloserine

Mohamed M. Shoukry and Afkar K. Abdel Hadi

Department of Chemistry, Faculty of Science, University of United Arab Emirates,
Al-Ain, P. O. Box 15551, United Arab Emirates

Received January 26, 1988

The complex-formation equilibria between copper(II), nickel(II), cobalt(II) and zinc(II) and cycloserine (D-4-amino-3-isoxazolidone, $C_3H_6O_2N_2$) were investigated potentiometrically. It was found that cycloserine forms 1:1 and 1:2 (metal ion:ligand) complexes. The ternary complex of Cu^{II} with nitrilotriacetic acid and cycloserine was investigated potentiometrically and spectrophotometrically. The probable bonding in the chelated compounds formed in aqueous solution was deduced. The formation constants follow the Irving-Williams series.

INTRODUCTION

Cycloserine (CS) has wide application in the pharmaceutical industry and is a drug commonly used in clinical therapeutics being a broad-spectrum antibiotic, inhibitory for *Mycobacterium tuberculosis*, *Escherichia coli*, *Staphylococcus aureus* and other microorganisms belonging to *Psittacosis-lymphogranuloma* group. It is known that the metal complexes of ligands which have biological activity are more active than the free ligands.^{1,2} The use of the drug as metal complex instead of the free molecule could represent a better route of administration associated with a small risk of toxic reactions and could be a good sustained-release pharmaceutical preparation. Copper(II) and palladium(II) complexes of cycloserine³ have been synthesized and characterized. However, no data on the complex-formation equilibria between transition metal ions, commonly existing in biological fluids, and cycloserine seems to have been reported.

We have a particular interest in the study of nitrilotriacetic acid (NTA).^{4,5} This stems from the resemblance of NTA coordination sites to those of proteins (e. g. Concanavalin).⁶ As a continuation of our research work oriented toward the study of binary and ternary complexes of biologically active ligands,⁷⁻¹² we present a further study. This investigation traces the formation and characteristics of binary and ternary complexes of cycloserine and nitrilotriacetic acid.

EXPERIMENTAL

Materials and Reagents

Cycloserine was provided by Fluka Chem. Co. Disodium salt of nitrilotriacetic acid was supplied by Merck. The metal salts were in the form of nitrate and

provided by BDH. All solutions were prepared in doubly distilled water. The metal ion solutions were standardized by EDTA using suitable indicators.¹³

Apparatus

The pH values were measured by means of 114D Pustl Munchen2 pH meter (Karl Kolb) using a special vessel described previously.⁴ The pH-meter and electrode were calibrated by using standard buffer solutions, prepared according to NBS specifications.¹⁴ The potentiometric titrations were carried out with a Metrohm 645 titrator. Spectrophotometric measurements were taken on a Pye Unicam SP8-400 spectrophotometer.

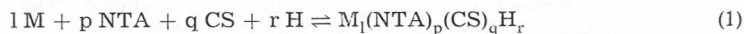
Procedure

The following mixtures were prepared for the determination of acid dissociation constant of cycloserine and formation constants of the binary and ternary complexes.

- (A) 10 mL of 0.02 M (CS) + 30 mL of 0.13 M KNO₃
 (B) 20 mL of 0.02 M (CS) + 10 mL of 0.02 M metal ion + 10 mL of 0.40 M KNO₃.
 (C) 10 mL of 0.02 M metal ion + 10 mL of 0.02 M (NTA) + 20 mL of 0.20 M KNO₃.
 (D) 10 mL of 0.02 M metal ion + 10 mL of 0.02 M (NTA) + 10 mL of 0.02 M (CS) + 10 mL of 0.40 M KNO₃.

Mixtures A—D were titrated against 0.20 M NaOH solution, at 25 °C in a purified N₂ atmosphere.

Formation constants, evaluated from titration data, are defined by Eq. 1 and 2



$$\beta_{lpqr} = \frac{[M_l(\text{NTA})_p(\text{CS})_q\text{H}_r]}{[M]^l[\text{NTA}]^p[\text{CS}]^q[\text{H}]^r} \quad (2)$$

Calculations were made with the aid of the MINQUAD-75 computer program¹⁵ on an IBM-4331 computer. The model selected was that which gave the best statistical fit and consistent with chemical logic. The formation constant, $K_{M(\text{NTA})\text{CS}}^{M(\text{NTA})}$, of the mixed-ligand complex was calculated assuming the complex M(NTA) is undissociated in the region of mixed-ligand complex formation. The results are listed in Table I.

TABLE I

Formation constants of metal ion complexes with cycloserine

System	l	p	q	r ^a	log β ^b	S ^c	χ _i ^{2 d}	R ^e
H ⁺	0	0	1	1	7.27(0.01)	1.0 × 10 ⁻⁷	6	0.006
	0	0	1	2	11.70(0.01)			
Cu ^{II}	1	0	1	0	6.03(0.03)	1.1 × 10 ⁻⁶	53	0.010
	1	0	2	0	9.93(0.03)			
	1	1	1	0	1.38(0.09)			
Ni ^{II}	1	0	1	0	3.99(0.03)	1.9 × 10 ⁻⁶	67	0.022
	1	0	2	0	6.52(0.06)			
Co ^{II}	1	0	1	0	3.10(0.03)	5.1 × 10 ⁻⁶	76	0.021
Zn ^{II}	1	0	1	0	3.31(0.01)	4.5 × 10 ⁻⁷	3	0.006
	1	0	2	0	5.60(0.01)			

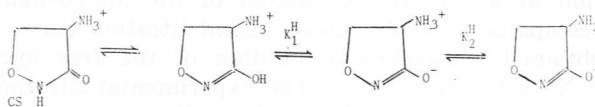
^a l, p, q and r are the stoichiometric coefficients corresponding to M^{II}, NTA, cycloserine and H⁺, respectively; ^b Standard deviations are given in parentheses; ^c Sum of square of residuals; ^d Chi square; ^e Hamilton's reliability factor.

Spectrophotometric measurements of the complexes Cu(NTA) , Cu(CS) , Cu(CS)_2 and Cu(NTA)CS were carried out. These complexes were prepared by mixing Cu^{II} and ligand solution in the appropriate ratio and the pH is raised to pH 7.5. The analytical concentration of Cu^{II} in these complexes was 0.004 M.

The charges on all the complex species reported in this paper are omitted for clarity.

RESULTS AND DISCUSSION

The potentiometric titration curve of cycloserine, mixture A, shows an inflection at $a = 1$, ($a = \text{moles of base added per mole of ligand}$), Figure 1. The addition of an equivalent of HNO_3 to cycloserine yields a potentiometric curve exhibiting inflections at $a = 1$ and $a = 2$. This could be ascribed to the proton dissociation from $(\text{N}=\text{COH})$ and (NH_3^+) groups according to the proposed equilibria



The binary complexes of cycloserine were investigated by titrating mixture (B). The potentiometric data of Cu^{II} , Ni^{II} and Zn^{II} complexes was fitted well by considering the formation of 1:1 and 1:2 complexes. The results of Co^{II} system revealed the formation of 1:1 complex only. The formation

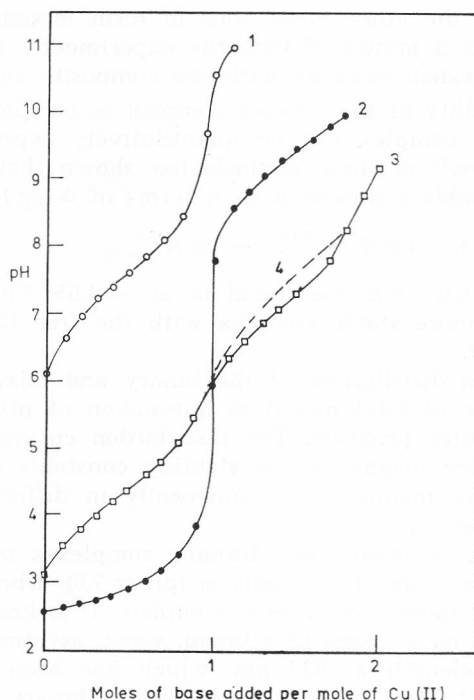


Figure 1. Potentiometric titration curves of Cu(II)-NTA cycloserine system. Curves: (1) Cycloserine; (2) Cu(II)-NTA ; (3) $\text{Cu(II)-NTA-cycloserine}$ and (4) Composite curve.

of protonated complexes with all metal ions was rejected. Therefore, the binding sites are the amino group and the exocyclic oxygen atom.

It is evident from Table I that the stability constants of the binary complexes follow the order



which is in accord with the Irving Williams order.¹⁶

The tendency of cycloserine to form mixed-ligand complexes with the above mentioned metal ions and NTA was studied. The titration curves of the Cu^{II} complexes are presented in Figure 1. The Cu^{II} -NTA (1:1) mixture titration curve starts at $\text{pH} \sim 2$ and has a low pH buffer region followed by a sharp inflection at $a = 1$, corresponding to complete formation of the 1:1 complex. The mixed Cu^{II} -NTA-cycloserine (1:1:1) solution does not show a sharp inflection at $a = 1$. The formation of the mixed-ligand complex is ascertained by comparison of the mixed ligand titration curve with the composite curve, obtained by graphical addition of the free cycloserine curve to the 1:1 Cu^{II} -NTA titration curve. The experimental titration curve of the mixed ligand system is quite different from the composite curve between $a = 1$ and $a = 2$, indicating the formation of the mixed-ligand complex.

Based on the above finding and in view of the fact that the 1:1 Cu^{II} -NTA complex is appreciably more stable¹⁷ than the 1:1 Cu^{II} -cycloserine complex, it can be assumed that in the presence of both ligands, the NTA will be primarily ligated to the Cu^{II} ion, with subsequent ligation of the cycloserine.

The tendency of the other metal ions to form mixed-ligand complexes seems rather poor. As a matter of fact, the experimental titration curves of their mixed ligand system coincide with the composite curve.

The relative stability of the ternary complex as compared to that of the corresponding binary complex can be quantitatively expressed in different ways. A recent review¹⁸ of these methods has shown that for a variety of reasons the most suitable comparison is in terms of $\Delta \log K$, given by Eq. 3.

$$\Delta \log K = \log K_{\text{Cu(NTA)CS}}^{\text{Cu(NTA)}} - \log K_{\text{Cu(CS)}}^{\text{Cu}} \quad (3)$$

The $\log K$ value of Cu^{II} ion was found to be -4.65 . This means that the cycloserine forms a more stable complex with the free Cu^{II} ion than with the Cu-NTA complex.

The concentration distribution of the binary and mixed-ligand complex species (as percentage of total metal) as a function of pH was obtained by MINQUAD-75 computer program. The distribution curves are presented in Figure 2. The different magnitudes of stability constants of the binary and ternary complexes are manifested unequivocally in different concentrations of the complex species.

Spectral analyses of binary and ternary complexes of the Cu^{II} system were performed under a specified condition ($\text{pH} = 7.5$), where it was assumed that the formation of these complexes is complete. It is known that the spectrum of aquated Cu^{II} ion consists of a broad, weak, asymmetric band ($\epsilon = 11$) with maximum wavelength at 833 nm, which has been attributed to the ${}^2T_{2g} \leftarrow {}^2E_g$ transition.¹⁹ The spectral bands of the binary and ternary Cu^{II} complexes are quite different from that of the aquated Cu^{II} ion, both as regards the positions of the maximum wavelengths and their average molar

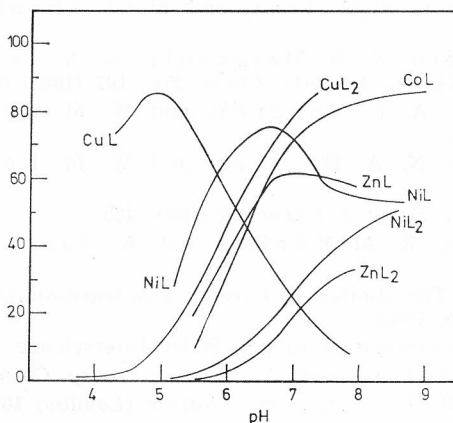


Figure 2. Concentration distribution of the complexes formed with cycloserine as a function of pH. The ordinate units: $100 \cdot [M]/[M]_{\text{tot}}$.

TABLE II

Maximum wavelengths of the spectral bands of Cu^{II} complexes

Cu^{II}	$T \times 10^3$		$\lambda_{\text{max}}/\text{nm}$	$\epsilon/\text{cm}^2 \text{ mmol}^{-1}$
	NTA	CS		
4	4	—	790	240
4	—	4	770	245
4	—	8	750	255
4	4	4	710	265

T = molar concentration.

absorptivities, Table II. There is a general hypsochromic shift with an increase in the spectral band intensity of the binary and ternary complexes, relative to that of the aquated Cu^{II} ion. It is noteworthy that hypsochromic shift, relative to that of aquated Cu^{II} ion, of 1:2 Cu^{II} -cycloserine is greater than that of the 1:1 complex. This is due to the increase in electron density in the vicinity of the metal ion d orbitals as second ligand molecule is added. The spectrum of the mixed-ligand complex exhibits a band at a much lower wavelength than those of $\text{Cu}(\text{NTA})$, $\text{Cu}(\text{CS})$ and $\text{Cu}(\text{CS})_2$. This confirms the formation of mixed-ligand complexes in solution.

REFERENCES

1. S. Krischner, Y. K. Wei, D. Francis, and J. G. Bergman, *J. Med. Chem.* **9** (1966) 369.
2. A. J. Thomson, R. J. P. Williams, and S. Reslova, *Structure and Bonding* **11** (1972) 1.
3. C. Preti and G. Tosi, *J. Coord. Chem.* **9** (1979) 125.
4. M. M. Shoukry, M. M. Khater, and E. M. Shoukry, *Indian J. Chem.* **25A** (1986) 488.
5. M. M. Shoukry and E. M. Shoukry, *Indian J. Chem.* **27A** (1988) 364.
6. K. D. Hardman, *Metal Ions in Biological Systems*, Plenum Press, New York, 1973.

7. D. L. Rabenstein, A. A. Isab, and M. M. Shoukry, *Inorg. Chem.* **21** (1982) 3234.
8. D. L. Rabenstein, S. A. Daignault, A. A. Isab, A. P. Arnold, and M. M. Shoukry, *J. Amer. Chem. Soc.* **107** (1985) 6435.
9. M. M. Shoukry, A. E. Mahgoub, and W. M. Hosny, *Transition Met. Chem.* **12** (1987) 77.
10. M. M. Shoukry, N. A. Darwish, and W. M. Hosny, *J. Chin. Chem. Soc.* **33** (1986) 179.
11. M. M. Shoukry, *Egypt J. Chem.* **28** (1985) 433.
12. M. M. Shoukry, E. M. Khairy, and A. Saeed, *J. Coord. Chem.* **17** (1988) 305.
13. F. J. Welcher, *The Analytical Uses of Ethylenediaminetetraacetic Acid*, Van Nostrand, Princeton, 1965.
14. R. G. Bates, *Determination of pH*, Wiley-Interscience, New York, 1973.
15. P. Gans, A. Sabatini, and A. Vacca, *Inorg. Chim. Acta* **18** (1976) 237.
16. H. Irving and R. P. Williams, *Nature (London)* **162** (1948) 746.
17. D. D. Perrin, *Stability Constants of Metal Ion Complexes*, Part B, Organic Ligands. Pergamon Press, 1979.
18. R. B. Martin and R. J. Prados, *J. Inorg. nucl. Chem.* **36** (1974) 1665.
19. B. N. Figgis, *Introduction to Ligands Fields*, Interscience, Publishers, New York, 1966.

SAŽETAK

Binarni i ternarni kompleksi cikloserina

M. M. Shoukry i A. K. Abdel Hadi

Metodom potenciometrijske titracije proučavano je nastajanje kompleksa bakra(II), nikla(II), kobalta(II) i cinka(II) s cikloserinom (D-3-amino-3-izoksolidom), i utvrđeno je da nastaju kompleksi sastava ML i ML₂. Ternarni kompleks bakra(II) s nitrilotriocetnom kiselinom i cikloserinom proučen je potenciometrijski i spektrofotometrijski, i izvedeni su zaključci o vjerojatnom načinu vezivanja u nastalim kelatima.