

Metal ion interaction with penicillins: Part VIII. Equilibrium study of mixed ligand complex formation of Co(II), Ni(II), Cu(II) and Zn(II) with ampicillin and some amino acids

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Abstract. Equilibrium study of complex formation of M^{2+} ions ($M = \text{Co}, \text{Ni}, \text{Cu}$ and Zn) with ampicillin (AH) and aminoacids (B), viz. glycinate, α -alaninate, β -alaninate and aspartate in ternary systems in aqueous solution at 37°C at a fixed ionic strength $I = 0.1 \text{ mol dm}^{-3}$ (NaNO_3) indicated the formation of mixed-ligand complexes of the types: $M(\text{A})(\text{B})$, $M(\text{A}-\text{H})(\text{B})$, $M(\text{A}-\text{H})(\text{B})(\text{OH})$, in addition to the binary $M(\text{A})$ and $M(\text{B})$ complexes. Stability of the mixed-ligand complexes were correlated with the modes of coordination of the ligands.

Keywords. Formation constants; mixed-ligand complexes; cobalt(II); nickel(II); copper(II); zinc(II); penicillins; ampicillin; aminoacidates.

1. Introduction

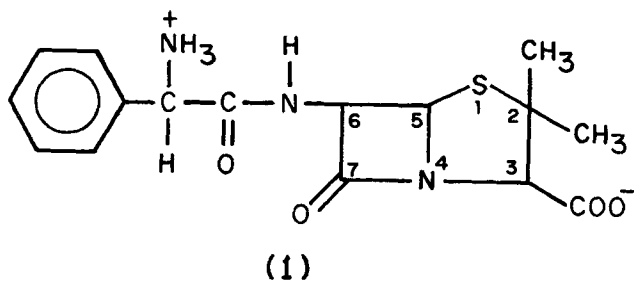
Antibiotic properties of ampicillin (1) are well-known (Florey 1949; Perlman 1970). A study on the interaction of ampicillin with biological metal ions in the presence of aminoacids as auxiliary ligands may provide useful information about the molecular mechanism of action of this drug. With a view to elucidate the varied mode of bonding of ampicillin, this paper describes a combined potentiometric and spectrophotometric study on the mixed ligand complex formation Co^{2+} , Ni^{2+} , Cu^{2+} and Zn^{2+} with ampicillin (AH) and aminoacids (B) viz., glycinate (Gly^-), α -alaninate ($\alpha\text{-Ala}^-$), β -alaninate ($\beta\text{-Ala}^-$) and aspartate (Asp^{2-}) in aqueous solution at 37°C at a fixed ionic strength, $I = 0.1 \text{ mol dm}^{-3}$ (NaNO_3).

2. Experimental

2.1 Materials and reagents

Ampicillin trihydrate ($\text{AH}\cdot 3\text{H}_2\text{O}$) (99%); MW, 403.46; m.p., 198–200 (decomposition); $[\alpha]_D^{20}$, $+247^\circ$ ($C = 1, \text{H}_2\text{O}$) obtained from Aldrich Chemical Company, Inc (USA) was directly used. All the other reagents were of AnalaR grade and their solutions were prepared in double distilled CO_2 free water. Freshly prepared solutions of ampicillin were always used. Metal ion solutions were standardized by ion-exchange

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separation combined with acid-base and complexometric titrations (Kolthoff *et al* 1967). Carbonate-free NaOH solution (Schwarzenbach and Biedermann 1948) was used as the titrant.

2.2 Equilibrium measurements

The following mixtures (initial volume 25 ml) were prepared for the equilibrium measurements.

- (i) 0.005 (M) HNO_3
- (ii) (i) + 0.001 ~ 0.002 (M) $\text{M}(\text{NO}_3)_2$
- (iii) (i) + 0.005 (M) AH_2^+
- (iv) (i) + 0.005 (M) BH_2^+ or BH_3^+ (B = Gly⁻, α -Ala⁻ and β -Ala⁻, or Asp²⁻)
- (v) (i) + 0.001 (M) AH_2^+ + 0.001 (M) $\text{M}(\text{NO}_3)_2$
- (vi) (i) + 0.005 (M) AH_2^+ + 0.001 (M) $\text{M}(\text{NO}_3)_2$
- (vii) (i) + 0.001 (M) BH_2^+ , or BH_3^+ + 0.001 (M) $\text{M}(\text{NO}_3)_2$
- (viii) (i) + 0.005 (M) BH_2^+ , or BH_3^+ + 0.001 (M) $\text{M}(\text{NO}_3)_2$
- (ix) (i) + 0.001 (M) AH_2^+ + 0.001 (M) BH_2^+ , or BH_3^+ + 0.001 (M) $\text{M}(\text{NO}_3)_2$

Required amounts of NaNO_3 were placed in each solution to have a fixed ionic strength, $I = 0.1 \text{ mol dm}^{-3}$ (NaNO_3). All the mixtures were allowed to attain equilibrium at the experimental temperature and were *pH* metrically titrated with a 0.1 (M) NaOH.

Ratios of $\text{M}^{2+}:\text{AH}:\text{BH}$ for *pH* metric titrations of ternary mixtures were kept at 1:1:1 to ensure the formation of only the simplest ternary complexes i.e., $\text{M}(\text{A})(\text{B})$ and $\text{M}(\text{A}-\text{H})(\text{B})$. For binary $\text{M}^{2+}:\text{AH}$ and $\text{M}^{2+}:\text{BH}$ systems, the metal:ligand ratios of 1:1 and 1:5 were maintained to provide scope for the formation of all the possible binary complexes. *pH* measurements were carried out with a systronics 335 *pH* meter (accuracy, $\pm 0.01 \text{ pH}$) employing a special glass electrode (*pH* 1–14) in conjunction with an SCE. pK_w of water at the experimental temperature was obtained from the literature (Wooleym *et al* 1970; Lurie 1978).

Electronic spectral measurements were carried out with a Cary 2390 spectrophotometer.

2.3 Calculation of formation constants

Proton–ligand constants and stability constants of binary and mixed ligand complexes were calculated with the aid of SCOGS computer programme (Sayce 1968, 1970; Sayce and Sharma 1972). Considering the deprotonation constants of the ligands and the hydrolysis constants of the M^{2+} (aq) ions (tables 1 and 2) the following species were

Table 1. Deprotonation constants^a of the ligands in aqueous solution^b.

Ligands	pK_3^H	pK_2^H	pK_1^H
Ampicillin (AH_2^+)	—	2.50	7.05
Glycine (BH_2^+)	—	2.14	9.42
α -Alanine (BH_2^+)	—	1.73	10.00
β -Alanine (BH_2^+)	—	2.45	10.65
Aspartic acid (BH_3^+)	1.60	3.69	9.60

^aLimits of error in the constants: ± 0.04 in log units;^bconditions: $I = 0.01 \text{ mol dm}^{-3}(\text{NaNO}_3)$; temperature = 37°C .**Table 2.** Hydrolysis constants^a (pK_M^H and pK_M^{2H}) of M^{2+} (aq) ions and stability constants^a [$\log K_{M(A)}^M$ and $\log K_{M(B)}^M$] of binary M^{2+} :AH and M^{2+} :B complexes in aqueous solution^b.

Constants	Metal ion				
	B	Co	Ni	Cu	Zn
pK_M^H		8.21	8.05	6.29	7.84
pK_M^{2H}		17.29	16.83	13.05	14.87
$\log K_{M(A)}^M$		3.21	3.66	4.79	2.98
$\log K_{M(B)}^M$	Gly ⁻	4.94	5.94	8.12	5.02
	α -Ala ⁻	4.12	5.34	7.52	4.49
	β -Ala ⁻	4.06	5.22	6.99	4.42
	Asp ²⁻	6.44	7.02	8.91	5.95

^aLimits of error: ± 0.04 in log units; ^bconditions: $I = 0.1 \text{ mol dm}^{-3}$ (NaNO_3); temperature = 37°C

considered to exist in the complexation equilibria: AH, A; BH_2 , BH, B, M(aq), M(OH), M(OH)₂, M(A), M(B), M(A)(OH), M(B)(OH), M(A)(B), M(A-H)(B), M(A-H)(B)(OH). Charges are omitted for clarity.

Mixed ligand constants are given in table 3. Complexation equilibria have been elucidated on the basis of the speciation curves (figure 1) obtained as computer outputs. Stabilities of ternary complexes have been characterised on the basis of $\Delta \log K_M$ values (Sigel 1975).

3. Results and discussions

Ampicillin in the monoprotonated form, AH_2^+ , behaves as a diprotic acid ($pK_{\text{COOH}}^H = 2.50$, $pK_{\text{NH}_3^+}^H = 7.05$). It forms complexes with M^{2+} ions ($M = \text{Co, Ni, Cu and Zn}$) at $pH > 3$, where the free ligand exists as a zwitterionic species, AH^\pm , and the coordinated ligand exists as a monoanion, (A^-) (Mukherjee and Ghosh 1991, 1994a).

Examination of the speciation curves of ternary $M^{2+}/AH/B$ systems with $M = \text{Co, Ni, Cu and Zn}$ (figure 1) indicates that the mixed-ligand M(A)(B) complexes with $B = \text{Gly}^-$

Table 3. Stability constants^a of mixed ligand complexes of $M^{2+}/AH/B$ systems in aqueous solution^b.

Constants	Amino acid				
	M	Gly ⁻	α -Ala ⁻	β -Ala ⁻	Asp ²⁻
$\text{Log } \beta_{M(A)(B)}^M$	Co	8.37	7.38	7.38	9.11
	Ni	9.99	9.10	8.73	10.28
	Cu	13.34	12.57	11.94	13.80
	Zn	8.19	7.57	7.48	8.44
$\Delta \text{Log } K_M$	Co	0.31	0.14	0.11	-0.45
	Ni	0.39	0.10	-0.15	-0.40
	Cu	0.43	0.26	0.16	0.10
	Zn	0.19	0.10	0.08	-0.49
$pK_{M(A)(B)}^H$	Co	9.60	—	9.60	9.65
	Ni	8.46	8.70	8.90	8.80
	Cu	8.34	8.23	8.38	8.75
$pK_{M(A-H)(B)(H_2O)}^H$	Ni	10.36	—	10.15	10.70
	Cu	10.29	9.96	9.92	10.20

^aLimits of error = ± 0.04 in log units; ^bconditions: $I = 0.10 \text{ mol dm}^{-3}$ (NaNO_3); temperature = 37°C .

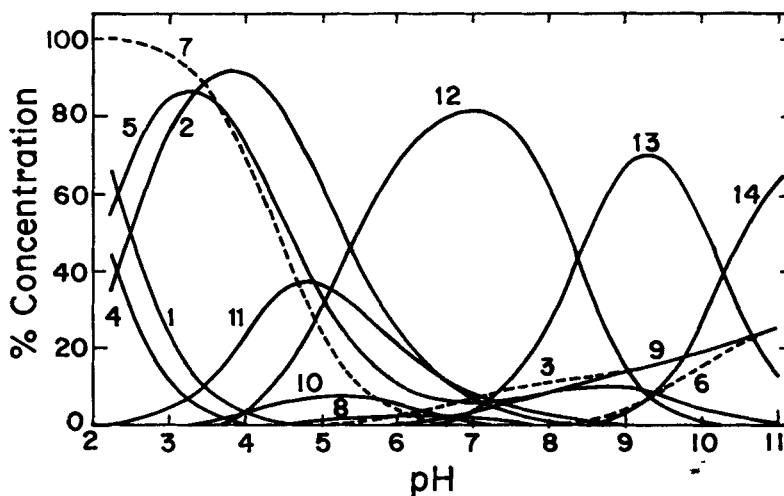
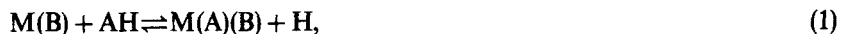


Figure 1. Speciation curves of $\text{Cu}^{2+}/\text{AH}/\text{glycinate}$ system at $37 \pm 0.1^\circ\text{C}$ (1) AH_2^+ , (2) AH^\pm , (3) A^- , (4) GlyH_2^+ , (5) GlyH^\pm , (6) Gly^- , (7) Cu^{2+} (8) $\text{Cu}(\text{OH})^+$, (9) $\text{Cu}(\text{OH})_2$, (10) $\text{Cu}(\text{A})^+$, (11) $\text{Cu}(\text{Gly})^+$, (12) $\text{Cu}(\text{A})(\text{Gly})$, (13) $\text{Cu}(\text{A-H})(\text{Gly})^-$, (14) $\text{Cu}(\text{A-H})(\text{Gly})(\text{OH})^{2-}$.

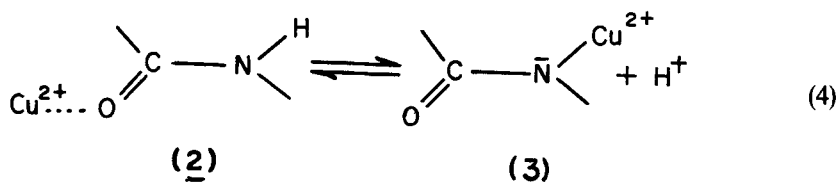
and Asp^{2-} are formed at $\text{pH} > 5$ according to the equilibria,



and those with $B = \alpha\text{-Ala}^-$ and $\beta\text{-Ala}^-$ are formed according to



A study of pH dependence of absorption maxima (λ_{\max}) of binary Cu^{2+} -ampicillin mixtures (Mukherjee and Ghosh 1991, 1994a) and correlation of the λ_{\max} values with the geometry of the amide ($-\text{CONH}-$) bonded copper(II) complexes (Sigel and Martin 1982; Gamp *et al* 1982) reveals (N, O) bidentate chelation by the ligand, A^- ion, in binary $\text{Cu}(A)$ and $\text{Cu}(A)_2$ complexes using the amino N- and amide carbonyl O-atoms. The complexes $\text{Cu}(A)$ and $\text{Cu}(A)_2$ appear as the predominant copper(II)-containing species at pH 5.0 and 7.7, and show λ_{\max} values at 730 and 670 nm respectively. At higher pH (> 8), the λ_{\max} values of these complexes are blue-shifted to 570 to 530 nm. The increase in the ligand field strength around the Cu^{2+} ion in these complexes is caused by the replacement of the amide carbonyl-oxygen atom by deprotonated amide- N^- atom, involving the transformation of A^- ion into $(A-H)^{2-}$ ion according to



Chelation by the amino-N and the deprotonated amide- N^- atoms of the $(A-H)^{2-}$ ion also places the thiazolidine-S atom at a coordinating position in the amide deprotonated $\text{Cu}(A-H)$ and $\text{Cu}(A-H)_2$ complexes. The relatively high intensity of the ${}^2E_g \rightarrow {}^2T_{2g}$ transition band of Cu^{2+} and its gradual merging with the charge transfer region with increase of pH of the solution is possibly caused by delocalization of the metal ion d_π electrons over to the vacant d_π orbitals of the S-atom. Terdentate (N, N^- , S) chelation by the $(A-H)^{2-}$ ions is also evident from relatively higher stability constants of $\text{Cu}(A-H)$ and $\text{Cu}(A-H)_2$ complexes as compared to those of the corresponding complexes derived from dipeptide ligands providing (N, N^-) bidentate coordination (Lim and Nancollas 1971; Yamauchi *et al* 1971). 1:1:1 $\text{Cu}^{2+} : \text{AH}$: glycine mixture shows a blue colour (λ_{\max} , 650 nm) at pH 5.0, where the concentration of the ternary $\text{Cu}(A)(\text{Gly})$ complex passes through a maximum (figure 1). This λ_{\max} value corresponds to a distorted octahedral geometry (4) for this complex $[\text{Cu}(\text{N}, \text{O})(\text{N}, \text{O})(\text{H}_2\text{O})_2]$ (Sigel and Martin 1982; Gamp *et al* 1982). The mixture shows a buffer region above pH 7. On adding one mole of base, the pH of the solution increases to 8.5 and its blue colour changes to violet (λ_{\max} , 570 nm). $\text{Cu}(A)(B)$ complexes with $B = \alpha\text{-Ala}^-$, $\beta\text{-Ala}^-$, and Asp^{2-} undergo similar blue-shifts in this pH range. Such a large blue-shift of the λ_{\max} (~ 80 nm) indicates an increase in the ligand field strength around Cu^{2+} , the only cause of which may be the metal-promoted deprotonation of the O-coordinated amide bond ($-\text{CONH}-$) in these complexes according to equilibrium (5) below, which transforms (N, O) bidentate A^- ion into (N, N^- , S) terdentate $(A-H)^{2-}$ ion within the coordination complex (5).

Metal-promoted amide deprotonation constants, $K_{M(A)(B)}^H$ could be calculated using,

$$\log K_{M(A)(B)}^H = \log \beta_{M(A-H)(B)}^M - \log \beta_{M(A)(B)}^M \quad (6)$$

On further rise of pH (> 9) the amide deprotonated $M(A-H)(B)$ complexes (5) derived from Ni^{2+} and Cu^{2+} show another buffer region, without any detectable spectral change.

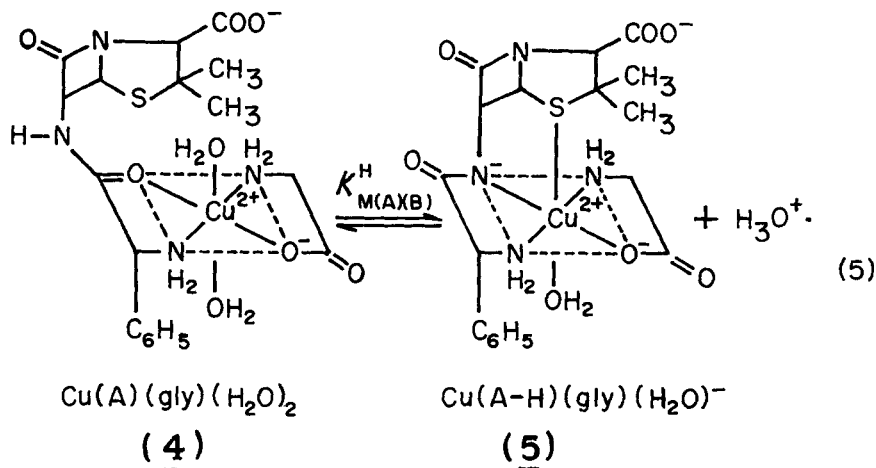
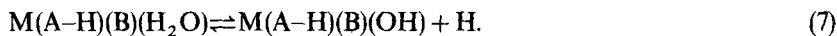


Table 3. Stability constants^a of mixed ligand complexes of $\text{M}^{2+}/\text{AH}/\text{B}$ systems in aqueous solution^b.

Constants	Amino acid				
	M	Gly ⁻	α -Ala ⁻	β -Ala ⁻	Asp ²⁻
$\text{Log } \beta_{\text{M(A)(B)}}^{\text{M}}$	Co	8.37	7.38	7.38	9.11
	Ni	9.99	9.10	8.73	10.28
	Cu	13.34	12.57	11.94	13.80
	Zn	8.19	7.57	7.48	8.44
$\Delta \text{Log } K_{\text{M}}$	Co	0.31	0.14	0.11	-0.45
	Ni	0.39	0.10	-0.15	-0.40
	Cu	0.43	0.26	0.16	0.10
	Zn	0.19	0.10	0.08	-0.49
$pK_{\text{M(A)(B)}}^{\text{H}}$	Co	9.60	—	9.60	9.65
	Ni	8.46	8.70	8.90	8.80
	Cu	8.34	8.23	8.38	8.75
$pK_{\text{M(A-H)(B)(H}_2\text{O)}}^{\text{H}}$	Ni	10.36	—	10.15	10.70
	Cu	10.29	9.96	9.92	10.20

^aLimits of error = ± 0.04 in log units; ^bconditions: $I = 0.10 \text{ mol dm}^{-3}$ (NaNO_3); temperature = 37°C .

This is obviously due to deprotonation of the coordinated H_2O molecule (Petitfaux *et al* 1970; Petitfaux 1973; Sjöberg 1973) in these complexes, according to



H_2O molecules are taken up by the M^{2+} ions in these complexes (5) for six-coordination. Replacing a coordinated H_2O molecule by an OH^- ion has very little effect on the ligand field strength around the metal ion (Mukherjee and Ghosh 1994b) as these two ligands are quite closely placed in the spectrochemical series (Jørgensen 1962). The coordinated water deprotonation constant, $K_{\text{M(A-H)(B)(H}_2\text{O)}}^{\text{H}}$, could be calculated using the relation

$$\text{log } K_{\text{M(A-H)(B)(H}_2\text{O)}}^{\text{H}} = \text{log } \beta_{\text{M(A-H)(B)(OH)}}^{\text{M}} - \text{log } \beta_{\text{M(A-H)(B)}}^{\text{H}} \quad (8)$$

Deprotonation of coordinated water in mixed-ligand complexes, $M(A-H)(B)(H_2O)$, to produce the quaternary complexes, $M(A-H)(B)(OH)$, is favoured due to $M(d_\pi) \rightarrow S(d_\pi)$ back bonding (Mukherjee and Ghosh 1991, 1994b, 1995). $pK_{M(A-H)(B)(H_2O)}^H$ values of coordinated water molecules are found to be much lower than the pK_w of water under the experimental conditions. This gives a measure of the extent of π -electron delocalization in the $M(d_\pi) \rightarrow S(d_\pi)$ bonds in these complexes.

Stability constants of the ternary $M(A)(B)$ complexes (table 3) fall in the Irving-Williams order (Irving and Williams 1948): $Co^{2+} < Ni^{2+} < Cu^{2+} > Zn^{2+}$ for all the four amino acid ligands. Steric hindrance by the CH_3 group possibly lowers the stabilities of the α -Ala⁻ complexes. Lower stabilities of the β -Ala⁻ complexes are the result of angle strain in the six-membered chelate ring without the double bond that is formed by this ligand.

$\Delta \log K_M$ values are found to be in the order: Gly⁻ > α -Ala⁻ > β -Ala⁻ > Asp²⁻, for the B ligands. Lower $\Delta \log K_M$ values for the Asp²⁻ complexes are possibly the results of bidentate (N, O⁻) coordination by the Asp²⁻ ion in the ternary $M(A)(Asp)$ complexes using its amino N-atom and the O-atom of one of the two carboxylate groups, leaving the other carboxylate group uncoordinated, although it provides terdentate (N, O⁻, O⁻) coordination in binary $M(Asp)$ complexes, using the amino N-atom and both the carboxylate O-atoms (Chaberek and Martell 1952). Decrease in the entropy arising from ordering of solvent molecules around the uncoordinated COO⁻ group of Asp²⁻ ion in the ternary $M(A)(Asp)$ and $M(A-H)(Asp)$ complexes may be responsible for lower effective values of the constants, $\beta_{M(A)(Asp)}^M$, and hence less favoured values of $\Delta \log K_M$ for the $M(A)(Asp)$ complexes. Tetrahedral geometry of Zn^{2+} is possibly not conducive to the terdentate (N, N⁻, S) mode of chelation by the amide deprotonated (A-H)²⁻ ion. As a result of this, all the ternary $Zn^{2+}/AH/B$ systems are found to be dominated by the hydroxo species.

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