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Solution Equilibria of Ni(II), Cu(II), and Zn(II) Complexes Involving Pyridoxine and Imidazole Containing Ligands: pH Metric, Spectral, Electrochemical, and Biological Studies

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Supporting Information

ABSTRACT: Solution equilibria and relevant stability constants for M(II)-pyridoxine (PN)(A) [M(II) = Cu(II), Ni(II), and Zn(II)], and M(II)-PN(A)-imidazole containing ligands (B) [B = imidazole (him), benzimidazole (bim), histamine (hist), and L-histidine (his)] in aqueous solution have been determined by a pH metric method at 310 K and I = 0.15 M $NaClO_4$. The complexation model for each system has been established by the MINIQUAD-75 program. The probable binding mode in the binary and ternary species was discussed based upon the derived stability constants. The concentration distributions of various species formed in solution were evaluated. The stability of ternary complexes follows the Irving—Williams order of metal ions, which was quantitatively ($\Delta \log K$, $\log X$, and % RS) compared with their corresponding binary complexes. In terms of the ligands (B), the stability of the complexes follows him > bim > hist > his. The molecular geometry of the complexes formed in solution between the ligands and M(II) was determined by electronic spectra at various pH intervals. The formation of complexes and their electrochemical properties were also assessed by cyclic voltammetry. The in vitro biological activity of the ternary complexes was tested against bacteria, fungi, and yeast.

INTRODUCTION

The vitamin B6 consists of three interrelated vitamins: pyridoxine, pyridoxal, and pyridoxamine. Pyridoxine (2-methyl-3-hydroxy-4,5-bis(hydroxymethyl)pyridine; Scheme 1), the first isolated vitamin B6 component, is an essential part of the human diet for the metabolism of amino acids and the maintenance of body cells. All three forms of vitamin B6 are converted into pyridoxal-5′-phosphate in the tissues, which exerts antiproliferative and antitumoral activities.[1] Pyridoxine analogues have demonstrated irreversible inhibition of thymidylate synthase.[2] Pyridine derivatives containing a hydroxy unit are present in anticancer agents.[3] The importance of metallic ions in some enzymatic processes involving vitamin B6 derivatives as cofactors is well-established.[4] Metal complexes of vitamin B6 have been reported[5] to inhibit the growth as well as the biosynthesis of RNA, DNA, and protein of *Escherichia coli* B-766. The imidazole group plays an important role in numerous bioactive compounds, and the imidazole ring was highly useful in pharmacology. It acts as a proton donor/acceptor and charge transfer agent and is ligated to the metal ions in the B12 coenzyme.[6]

Pyridoxine displays different coordination sites with metal ions with different charges and hard/soft character. Chelation through phenolate oxygen and adjacent hydroxymethyl groups is common for pyridoxine metal complexes.[7−10] Other bonding modes of pyridoxine are (i) simple coordination through the pyridine nitrogen,[7] (ii) chelation plus bonding through the pyridine nitrogen,[11−13] and (iii) chelation plus bridging through the coordinated phenolate or hydroxyl group.[14,15] Though considerable attention has been devoted to the study of vitamin B6 components,[16−18] little attention has been paid to pyridoxine containing ternary complexes.

In continuation of our earlier works on amino acid ternary complexes,[19−23] we report here the solution equilibria and stability of ternary complexes of Ni(II), Cu(II), and Zn(II) with pyridoxine (PN) in the presence of imidazole containing ligands, namely, imidazole (him), benzimidazole (bim), histamine (hist), and L-histidine (his). pH metric, spectrophotometric, and cyclic voltammetric studies on these systems were carried out. The studies have helped in understanding the coordination environment of pyridoxine in presence of other ligand(s) around the metal ion. Biological activities of these complexes with different microorganisms were also evaluated.

EXPERIMENTAL SECTION

Materials. All of the ligands used in this study were extra pure products (Fluka AG, pure 99.9 %). All other chemicals used were of reagent grade purity. Metal perchlorates and other reagents were prepared and estimated as described elsewhere.[19−25] The dilute solutions were prepared from doubly distilled deionized water which was previously boiled to remove dissolved CO_2. Alkali titer concentrations and the absence of carbonate were periodically checked by means of the appropriate Gran titrations[26] against potassium hydrogen phthalate. The concentration of sodium perchlorate (Fluka AG) was checked by the

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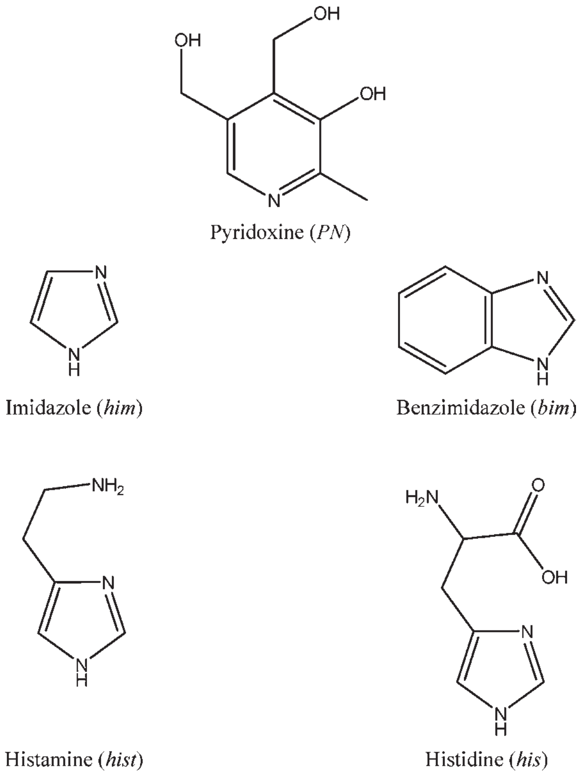
Scheme 1



estimation of perchloric acid obtained when a known volume of diluted sodium perchlorate was passed through Amberlite IR 120 (H^+) resin column.

pH Metric Measurements. The pH titrations were carried out at 310 K in a digital pH meter (Systronics μ pH System 361) with an extension of a combined glass electrode (accuracy ± 0.01 pH unit). The ionic strength of each solution was adjusted to 0.15 M with $NaClO_4$ as the supporting electrolyte. In both the acidic and alkaline regions, the electrode system was calibrated in terms of hydrogen ion concentration instead of activities. The calibration of the electrode system was done by direct titration of a dilute solution of perchloric acid using sodium perchlorate background and also by recognized buffer solutions in terms of concentration by the method of Irving et al.[26] The ionic product of water (pK_w) is -13.62 at 310 K. Multiple titrations were carried out for each system. Oxygen-free nitrogen gas was bubbled through the solution before and during titrations. The protonation constants for pyridoxine were obtained from its solutions of concentration ranging from ($3.0 \cdot 10^{-3}$ to $1.5 \cdot 10^{-2}$) M. In the binary systems 50 mL solutions containing low concentration of metal perchlorate and the ligands (1:1, 1:2, and 1:5) were used. In the ternary systems M(II), ligand (A), ligand (B), and H^+ ions were chosen as components in evaluating the stability constants. The stability constant values were evaluated with the aid of the MINIQUAD-75 program.[27,28] The concentration distribution profiles were obtained[29] with HYSS.

UV–vis Measurements. Spectrophotometeric determinations were performed with a Shimadzu 2450 (cell length, 1 cm) using water as the solvent at 310 K. Complexation equilibria for binary complexes (1:1, 1:2, and 1:5) and for ternary complexes (1:1:1 and 1:2:2) in the range (400 to 1100) nm were studied by means of the spectra of five to seven solutions at appropriate pH. The pH for maximum complexation of a particular species has been obtained from a species distribution diagram.

Table 1. Stability Constants for the Proton and Parent Binary Complexes of Ni(II), Cu(II), and Zn(II) with PN(A) at 210 K, $I = 0.15$ M ($NaClO_4$) (Standard Deviations Are Given in Parentheses)

parameter	Ni(II)	Cu(II)	Zn(II)
$\log \beta_{HA}$	8.77(4)	8.77(4)	8.77(4)
$\log \beta_{H_2A}$	13.21(3)	13.21(3)	13.21(3)
$\log \beta_{MA}$	4.80(4)	5.46(3)	4.53(4)
$\log \beta_{MA_2}$	9.88(5)	10.74(6)	9.24(6)
$\log K_{MA2}^{MA}$	5.08	5.28	4.71
$\log K_1/K_2$	0.49	0.51	0.49

Cyclic Voltammetric Measurements. Electrochemical behavior of the ligand, binary, and ternary complexes was examined by means of cyclic voltammetry employing glassy carbon as the working electrode, Ag/AgCl as reference electrode and platinum wire as auxiliary electrode. The working media consisted of DMSO solution of metal perchlorate, pyridoxine and imidazole containing ligands (1:1:1 and 1:2:2) and $[Bu_4N]ClO_4$ as supporting electrolyte. The solution was degassed with N_2 prior to use and kept under N_2 atmosphere throughout the experiment. All of the measurements were carried out using 3 mM solutions at room temperature in the potential range (-1.5 to $+1.5$) V with a scan rate ranging from 25 $mV \cdot s^{-1}$ to 100 $mV \cdot s^{-1}$.

Biological Studies. The in vitro biological activity of the ternary complexes was tested against the gram positive bacteria (*Staphylococcus aureus*) and gram negative bacteria (*Pseudomonas aeruginosa, Escherichia coli, Proteus species, Klebsiella species*), fungi (*Aspergillus niger*), and yeast (*Candida species*) by a modified disk diffusion method.[30] The bacteria were cultured for 24 h at 310 K in an incubator. The test solutions containing M(II)-PN-him/bim/hist/his (1:1:1) [$3 \cdot 10^{-3}$ M concentration] in water as solvent were added dropwise to a 10 mm diameter Whatman No. 1 filter paper disk placed at the center of each agar plate. These discs were then kept at 278 K for 1 h and then transferred to an incubator maintained at 310 K. After 3 days, the inhibition zone appearing around the discs in each plate was measured. Commercially available ampicillin was used as antibacterial control, while nystatin was used as antifungal control.

RESULTS AND DISCUSSION

Protonation Constants and Binary Complex Equilibria. The stepwise protonation constants of PN have been determined using pH metric titrations (Table 1). Though PN has four protonation sites (Scheme 1), the present experimental conditions identified only two protonation centers, namely, the phenolate oxygen ($\log K_1 = 8.77$) and the hydroxymethyl oxygen ($\log K_2 = 4.44$). There is a gap of 4.33 log units for the second proton addition in PN system. The investigation of complex formation between PN(A) and metal ions was carried out using pH metric titrations. For all of the metal ions, precipitation occurred at a higher pH (>7.5). Analysis of the data (M:A = 1:1; 1:2, and 1:5) using the MINIQUAD-75 program revealed MA and MA_2 stoichiometries for Ni(II), Cu(II), and Zn(II)-PN(A) complexes. There is no evidence of complex formation at low pH,

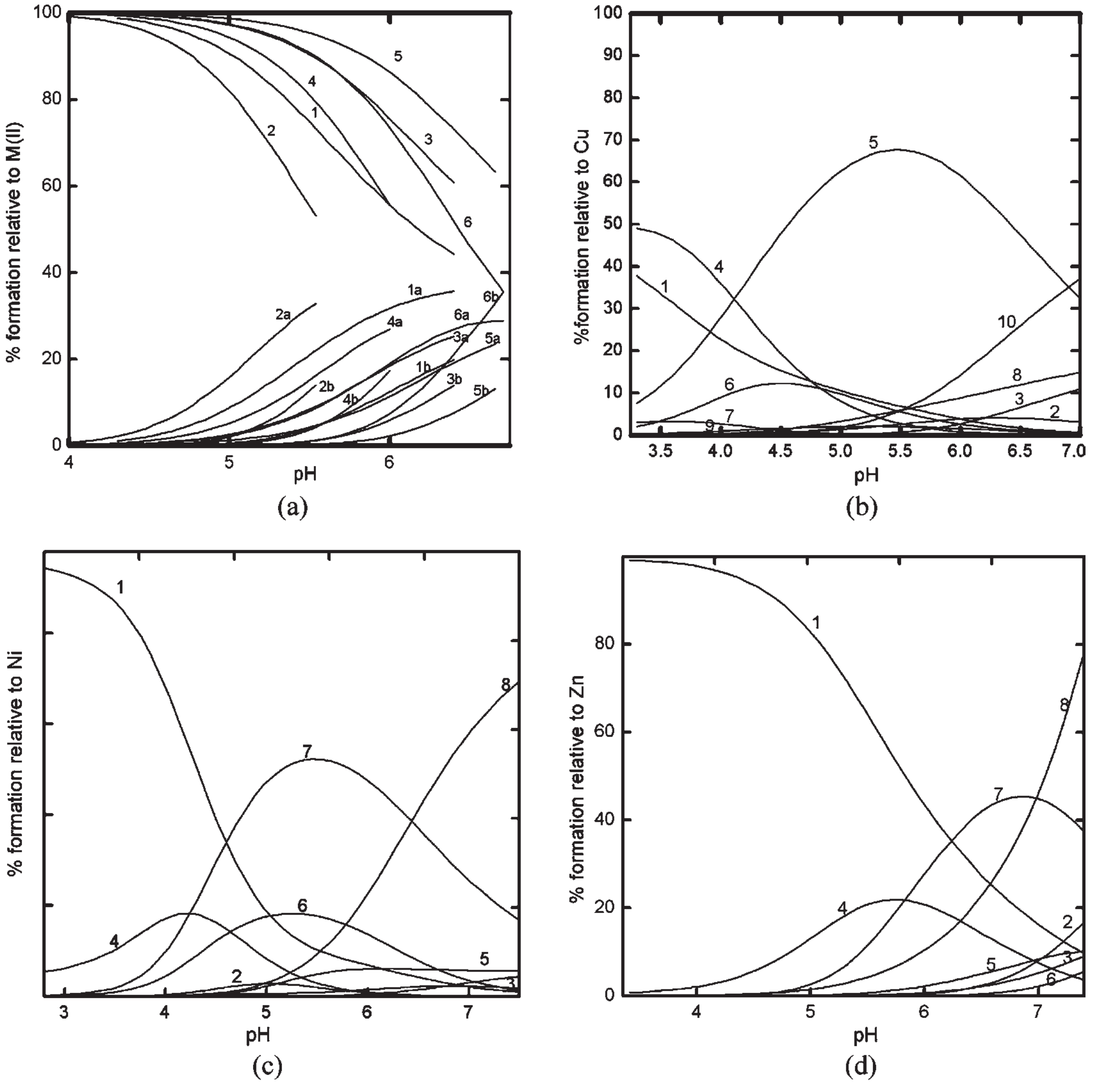


Figure 1. Species distribution diagram for (a) M(II)-PN(A), [M(II) = Ni(II), Cu(II), and Zn(II)], where 1:1: 1, free Cu; 1a, CuA; 1b, CuA_2; 1:2: 2, free Cu; 2a, CuA; 2b, CuA_2; 1:1: 3, free Ni; 3a, NiA; 3b, NiA_2; 1:2: 4, free Ni; 4a, NiA; 4b, NiA_2; 1:1: 5, free Zn; 5a, ZnA; 5b, ZnA_2; 1:2: 6, free Zn; 6a, ZnA; 6b, ZnA_2; (b) Cu(II)-PN(A)-his(B): 1, free Cu; 2, CuA; 3, CuA_2; 4, CuBH; 5, CuB; 6, CuB_2H; 7, CuB_2H_2; 8, CuB_2; 9, CuABH; 10, CuAB; (c) Ni(II)-PN(A)-his(B): 1, free Ni; 2, NiA; 3, NiA_2; 4, NiBH; 5, NiB; 6, NiB_2; 7, NiABH; 8, NiAB; (d) Zn(II)-PN(A)-his(B) systems $C_M = 0.003$, $C_A = C_B = 0.003$ M: 1, free Zn; 2, ZnA; 3, ZnA_2; 4, ZnBH; 5, ZnB; 6, ZnB_2; 7, ZnABH; 8, ZnAB.

and the species distribution curves showed appreciable complex formation from pH 5.0 and maximum at higher pH values too close to the point of precipitation. The precipitates observed during the titrations are ascribed to hydrolysis products. The value of log K_1/K_2 is ~0.5 (Table 1), which reveals the formation of MA_2 species. The maximum percentage of concentration for MA and MA_2 species were ~35% and ~15%, respectively, at pH 6.5 (Figure 1). The M(II)-him, bim, hist, and his(B) complexation equilibria have been taken from the literature.[19−22]

The complexation between metal ions and PN was followed by measuring the absorption spectra of aqueous solution containing Ni(II)/Cu(II)/Zn(II) perchlorate and PN in the ratio of 1:1; 1:2, and 1:5 in the pH range 4 to 7. The electronic spectra show that the wavelength of maximum absorption at pH 4.0 is already lower than that corresponding to the aqueous M(II) ions. As pH increases, the intensity of maximum absorption increases and continuously shifts to lower wavelength, demonstrating the complexation of PN with metal ions. In the UV region the PN ligand shows an absorption for the heterocycle at 277 nm ($\varepsilon = 21\,325$ $dm^{-3} \cdot mol^{-1} \cdot cm^{-1}$). Solutions containing M(II) and increasing amounts of PN at high pH values in the ratio of 1:1; 1:2, and 1:5 show no appreciable change in the absorption due to heterocycle but show a marked decrease in the absorbance [$\varepsilon = 17\,239$ for Ni(II), $\varepsilon = 17\,546$ for Cu(II); and $\varepsilon = 18\,334$ $dm^{-3} \cdot mol^{-1} \cdot cm^{-1}$ for Zn(II) complexation in the 1:2 ratio]. This clearly demonstrates the noninvolvement of the pyridine nitrogen in coordination with the metal ion. Electronic spectra of the M(II)-PN(A) complexes exhibit only one isosbestic point at 665 nm corresponding to the MA/MA_2 equilibrium in the pH range 3.0 to 7.0. It is likely that, in the M(II)-PN complexes, the metal ion is coordinated to phenolic and hydroxymethyl oxygen atoms forming a six-membered ring.

Ternary Complex Equilibria. In the formation of ternary complexes, the general four-component equilibria can be written

Table 2. Stability Constants for the Proton and Parent Binary Complexes of Ni(II), Cu(II), and Zn(II) with him, bim, hist, and his(B) Complexes[19−22] at 310 K, I = 0.15 M ($NaClO_4$) (Standard Deviations Are Given in Parentheses)

	Ni(II)-B				Cu(II)-B				Zn(II)-B			
parameter	him	bim	hist	his	him	bim	hist	his	him	bim	hist	his
$\log \beta_{HB}$	6.55(2)	5.68(1)	9.39(8)	8.96(3)	6.95(2)	5.68(1)	9.39(8)	8.96(3)	6.95(2)	5.68(9)	9.39(8)	8.96(3)
$\log \beta_{H_2B}$	–	–	15.34(1)	14.96(5)	–	–	15.34(1)	14.96(5)	–	–	15.34(1)	14.96(5)
$\log \beta_{H_3B}$	–	–	–	17.37(9)	–	–	–	17.37(9)	–	–	–	17.37(9)
$\log \beta_{MBH}$	–	–	11.85(3)	13.08(5)	–	–	13.46(4)	14.38(4)	–	–	12.21(5)	11.43(5)
$\log \beta_{MB}$	3.37(5)	2.37(4)	6.36(2)	8.84(7)	4.21(5)	3.44(4)	9.24(18)	10.27(2)	2.98(3)	1.93(3)	5.25(5)	6.48(4)
$\log \beta_{MB_2H}$	–	–	17.86(4)	21.04(7)	–	–	21.82(6)	23.96(2)	–	–	–	17.81(5)
$\log \beta_{MB_2}$	5.70(9)	4.11(9)	11.22(2)	15.94(4)	7.55(9)	6.29(9)	16.16(4)	18.49(4)	5.63(4)	4.38(5)	10.21(6)	12.07(6)
$\log \beta_{MB_3}$	7.90(9)	–	–	–	10.73(9)	8.70(9)	–	–	–	–	–	–
$\log \beta_{MB_4}$	9.57(11)	–	–	–	12.91(1)	10.87(9)	–	–	–	–	–	–

Table 3. Stability Constants and Stabilization Effect of the Ni(II)-PN(A)-him, bim, hist, and his(B) Ternary Complex Systems at 310 K, I = 0.15 M ($NaClO_4$) (Standard Deviations Are Given in Parentheses)

	Ni(II)-B				Cu(II)-B				Zn(II)-B			
parameter	him	bim	hist	his	him	bim	hist	his	him	bim	hist	his
$\log \beta_{MABH}$	–	–	17.69(5)	19.83(6)	–	–	19.76(5)	21.13(2)	–	–	17.85(3)	18.29(4)
$\log \beta_{MAB}$	8.36(1)	7.53(3)	11.54(3)	14.06(5)	9.99(1)	9.3(3)	15.21(1)	16.39(3)	7.55(2)	6.95(4)	10.29(2)	11.68(5)
pK^{H}_{MABH}	–	–	6.15	5.77	–	–	4.55	4.74	–	–	7.56	6.61
$\log K^{MA}_{MAB}$	3.56	2.73	6.74	9.26	4.53	3.84	9.75	10.93	3.02	2.42	5.76	8.15
$\log K^{MB}_{MAB}$	4.99	5.16	5.18	5.22	5.78	5.86	5.97	6.12	4.57	5.02	5.04	5.20
$\log K^{MA}_{MABH}$	–	–	12.89	15.03	–	–	14.30	15.67	–	–	13.32	13.76
$\log K^{MBH}_{MABH}$	–	–	5.84	6.75	–	–	6.30	6.75	–	–	5.64	6.86
$\Delta \log K_{MAB}$	0.19	0.36	0.38	0.42	0.32	0.40	0.51	0.66	0.04	0.49	0.51	0.67
$\log X$	1.14	1.07	1.98	2.30	1.49	1.55	3.50	3.53	0.23	0.28	1.13	2.05
$\log X'$	0.09	0.08	0.10	0.14	0.48	0.56	0.67	0.82	0.14	0.31	0.33	0.49
$\Delta \log_{MABH}$	–	–	1.04	1.95	–	–	0.84	1.29	–	–	1.11	2.15
% RS	5.64	15.19	5.97	4.75	7.60	11.63	5.52	6.43	1.34	25.39	9.71	25.77

as follows (charges being omitted for the sake of simplicity):

$$pM + qA + rB + sH \rightleftharpoons M_pA_qB_rH_s$$

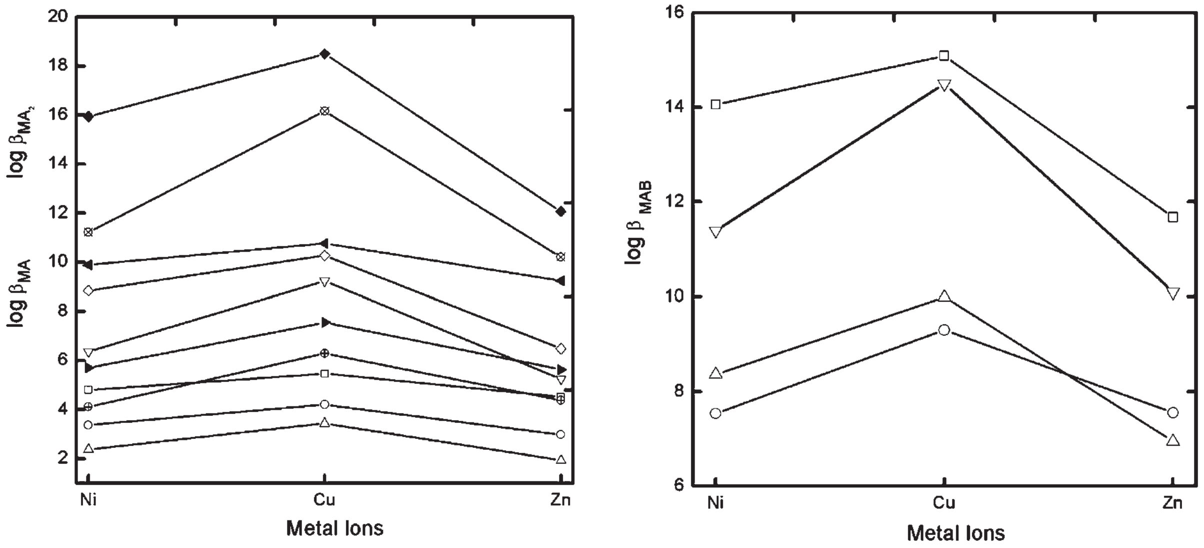
where p, q, r, and s are stoichiometric coefficients of M(II), PN(A), imidazole containing ligands (B), and H, respectively.

The overall stability constant corresponding to the equilibrium is defined as

$$\beta_{pqrs} = \frac{[M_pA_qB_rH_s]}{[M]^p[A]^q[B]^r[H]^s}$$

Many sets of ternary species for each system were examined using the MINIQUAD-75 program.[27,28] The best fit model was selected on the basis of chi square (χ^2), the reliability factor (R), and the sum of the squared residuals (S). Ternary complexes of Ni(II) and Zn(II) were studied in the pH range 3.0 to 7.5, while Cu(II) complexes were 4.0 to 7.0. The M(II)-PN(A)-him/bim(B) systems showed the presence of MAB species, while in M(II)-PN(A)-hist/his(B) systems an additional MABH species has also been detected (Table 3). The species distribution diagram provides a clear picture of distribution of metal ions among two different ligands. Under the present experimental conditions, the amount of MABH species is in the range of (20 to 60) % of the total metal ion concentrations. The protonated species MABH was found to be favored above pH 4.0 in all of the systems and is in larger concentration, 60 %, in Ni(II) systems. The MAB species prevails at pH 6.5 with a degree of formation ranging from (65 to 90) %. Also, in the 1:2:2 solution the concentration of the MAB species is more than in the 1:1:1 solution (Figure 1).

The pK^{H}_{MABH} values obtained in the M(II)-PN(A)-hist(B) and his systems are not comparable to each other but follow the trends of the respective pK^{H}_{MBH} values in the M(II)-hist and his(B) systems (Tables 2 and 3). This indicates that the extra proton of MABH species in the hist and his(B) systems is attached with the ligand (B). The structure of the protonated his complex, MBH, has been interpreted in many ways, namely, (i) protonation of the amino group and imidazole nitrogen coordinates with the metal ion (leaving the carboxyl group free) and (ii) protonation of the amino group and imidazole-N and carboxyl-O coordinate with the metal ions. In the present study, the $\log K^{MA}_{MABH}$/$\log K^{MBH}_{MABH}$ values calculated compare favorably with $\log K^{M}_{MBH}$ and $\log K^{M}_{MA}$ values, respectively. The $\log K^{M}_{MBH}$ value is taken from our previous study,[20] which is calculated on the basis of



(a) (b)

Figure 2. (a) Relation between log β_{MA} and log β_{MA2} for PN, him, bim, hist, and his with Ni(II), Cu(II), and Zn(II) complexes and atomic number of the metal used: □, PN; △, him; ○, bim; ▽, hist; ◇, his; for log β_{MA} and ■, PN; ▲, him; ⊕, bim; ⊗, hist; ♦, his; for log β_{MA2} values. (b) The relation between the log β_{MAB} for △, PN:him; ○, PN:bim; ▽, PN:hist; and □, PN:his; with Ni(II), Cu(II), and Zn(II) complexes and atomic number of the metal used.

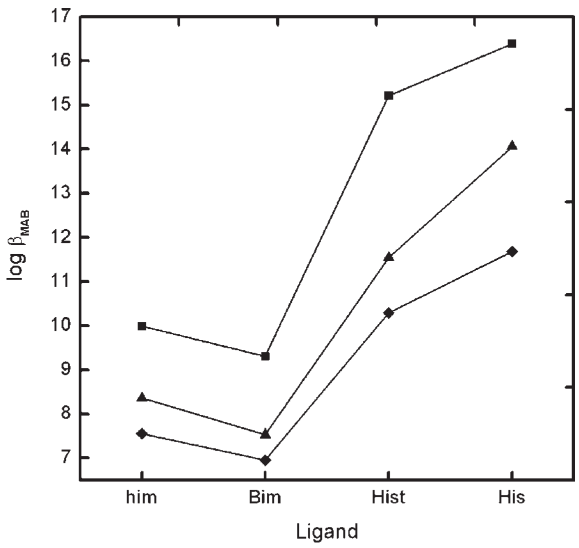


Figure 3. Relation between the logarithms of the stability constants, log β_{MAB} for PN:him, bim, hist, and his with secondary ligands [■, CuAB; ▲, NiAB; and ♦, ZnAB].

point (ii) noted above. Thus, the formation of MABH species results from

$$\mathrm{MA} + \mathrm{MBH} \rightleftharpoons \mathrm{MABH}$$

$$\beta_{\mathrm{MABH}} = \frac{[\mathrm{MABH}]}{[\mathrm{MA}][\mathrm{MBH}]}$$

The stabilities of the binary and ternary complexes can be compared using the Δ log K parameter. The Δ log K_{MABH} [= log $\beta_{MABH} - (\log \beta_{MA} + \log \beta_{MBH})$] values computed in all of these systems are positive compared to the statistically expected values[31] suggesting that MABH species are markedly stabilized.

The formation of MAB species has been identified in all the investigated systems. In the MA binary species, PN coordinates the metal through the phenolic oxygen and hydroxy methyl oxygen atoms. The same type of binding can be expected in the MAB species in the M(II)-PN(A)-him/bim(B) systems, because log K^{MB}_{MAB} values obtained (Table 3) bear favorable comparison with the log K^{M}_{MA} value obtained in the M(II)-PN(A) system (Table 1). The log K^{MA}_{MAB} values (Table 3) on these systems bear a favorable comparison with the corresponding log K^{M}_{MB} values in the M(II)-him/bim(B) systems (Table 2) suggesting monodentate binding of him/bim in the ternary species. Thus, in MAB species (B = him/bim), three coordination positions would be occupied by the bidentate binding of PN and the monodentate binding of him/bim ligand, and the remaining position would be completed by a solvent water molecule. The log K^{MB}_{MAB} and log K^{MA}_{MAB} values for the M(II)-PN(A)-hist(B) system correspond to the bidentate binding of both PN and hist ligands, giving a coordination number of four to the metal ion in the MAB species. The log β_{MAB} value obtained in the M(II)-PN-his systems (B = his) is higher than that of M(II)-PN(A)-hist systems (B = hist). This suggests that, in the MAB species, his (B) binds the metal ion in a tridentate manner.

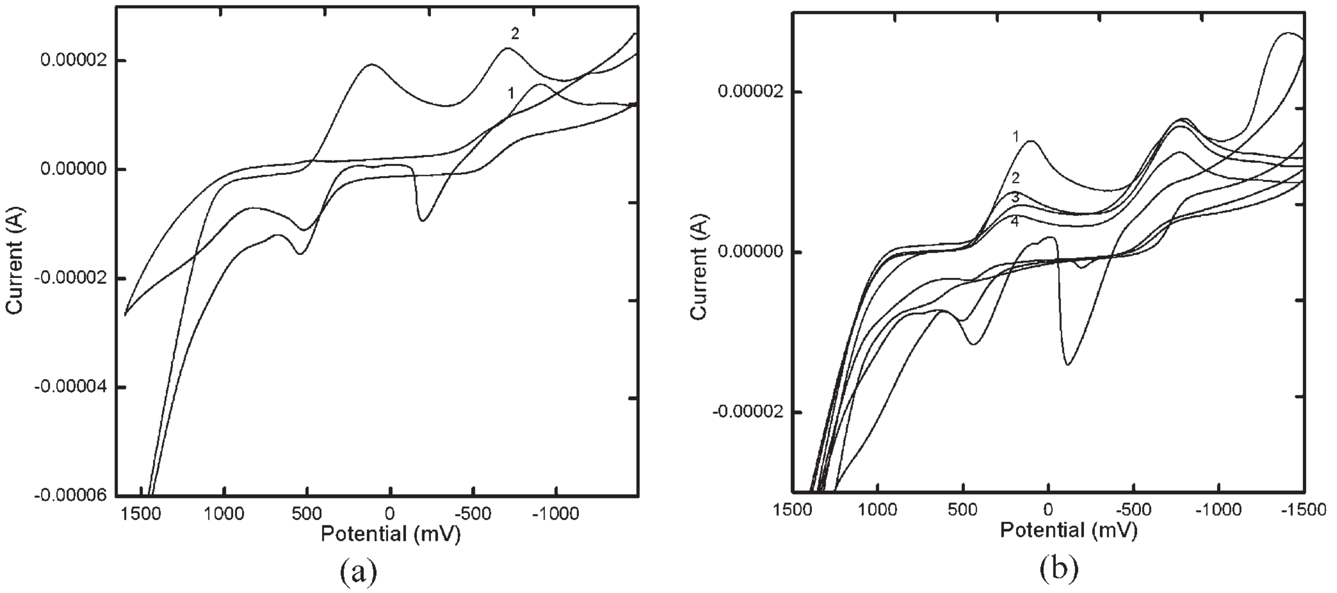
The relative stabilities of ternary complexes and the corresponding binary complexes can be expressed quantitatively[31−34] in terms of Δ log K, % RS, and log X. The Δ log K_{MAB} values calculated for the title systems are more positive than the statistically expected value[31,32] indicating that the secondary ligand adds on to the MA binary complex rather than to the aquated metal ion (MA + B ⇌ MAB). Δ log K values measure the relative ease or difficulty in the formation of the ternary complexes. But Δ log K values cannot be compared for different ternary complexes. The relative stabilization (% RS) is used for this purpose (Table 3).

$$\%\mathrm{RS} = [(\log K^{MA}_{MAB} - \log K^{M}_{MB})/\log K^{M}_{MB}]\cdot 100$$

The log X value indicates the coordination ability of secondary ligand toward MA, and the statistically expected value is 0.6 for all

Table 4. Electronic Absorption Spectral Data of M(II)-(A)-(B) Ternary Complexes [M] = [A] = [B] = $3 \cdot 10^{-3}$ M at 310 K in DMSO at pH = 4 to 7

complexes	absorption λ_{max} (in nm)	band assignments	B	β	geometry
Cu(II)-PN-him	392	INCT		-	distorted
	795	$^2T_2 \rightarrow {}^2E_2$	-		tetrahedral
Cu(II)- PN-bim	422	INCT	-	-	distorted
	756	$^2T_2 \rightarrow {}^2E_2$			tetrahedral
Cu(II)-PN-hist	829	$^2T_2 \rightarrow {}^2E_2$	-	-	distorted tetrahedral
Cu(II)-PN-his	329	INCT	-	-	
	at pH 6.5, 690	$^2E_2 \rightarrow {}^2T_2$			distorted octahedral
	at pH 5.5, 782	$^2T_2 \rightarrow {}^2E_2$			tetrahedral
Ni(II)-PN-him	393	$^3A_{2g}$ (F)$\rightarrow {}^3T_{1g}$ (P)			distorted
	652, 721	$^3A_{2g}$ (F)$\rightarrow {}^3T_{1g}$ (F)	897	0.87	octahedral
	1098	$^3A_{2g}$ (F)$\rightarrow {}^3T_{1g}$ (F)			
Ni(II)-PN-bim	393	$^3A_{2g}$ (F)$\rightarrow {}^3T_{1g}$ (P)			distorted
	655, 721	$^3A_{2g}$ (F)$\rightarrow {}^3T_{1g}$ (F)	897	0.87	octahedral
	1100	$^3A_{2g}$ (F)$\rightarrow {}^3T_{1g}$ (F)			
Ni(II)-PN-hist	394	$^3A_{2g}$ (F)$\rightarrow {}^3T_{1g}$ (P)			distorted
	657, 721	$^3A_{2g}$ (F)$\rightarrow {}^3T_{1g}$ (F)	882	0.86	octahedral
	1096	$^3A_{2g}$ (F)$\rightarrow {}^3T_{1g}$ (F)			
Ni(II)-PN-his	394	$^3A_{2g}$ (F)$\rightarrow {}^3T_{1g}$ (P)			distorted
	646, 732	$^3A_{2g}$ (F)$\rightarrow {}^3T_{1g}$ (F)	692	0.67	octahedral
	1058	$^3A_{2g}$ (F)$\rightarrow {}^3T_{1g}$ (F)			



(a)

(b)

Figure 4. Cyclic voltammograms of (a) 1, PN; 2, Cu(II)-PN; and (b) 1, Cu(II)-PN-him; 2, Cu(II)-PN-bim; 3, Cu(II)-PN-hist; 4, Cu(II)-PN-his in DMSO solution. Scan rate (50 mV·s^{-1}) at 310 K.

geometries.[32] The calculated log X values are higher than the statistical value indicating marked stabilization of ternary complexes. But, the log X value fails to throw any light on the stabilizing order of metal ligand bonds after the complex formation. Hence, $\Delta \log X'$ is considered.[34]

$$\Delta\log X' = (\log \beta^{MA}_{MAB} + \log K^{M}_{MA}) - (\log \beta_{MA_2} + \log K^{M}_{MB})$$

The statistically expected $\Delta \log X'$ value is 0.3. In the present studies, the data reveal that log X' values are of the order of statistical values indicating stabilization of ternary complexes compared to other species. From the stability constant values calculated, it has been observed that the binary and ternary complexes follow the Irving–Williams order with respect to metal ions (Figure 2).[35] Also, the overall stability constant value of the same metal ion with respect to the imidazole containing ligand is of the order (Figure 3):

M(II)-PN-his > M(II)-PN-hist > M(II)-PN-him > M(II)-PN-bim

The higher stability for the ternary complexes in the him(B) systems compared to those in the bim(B) systems may be

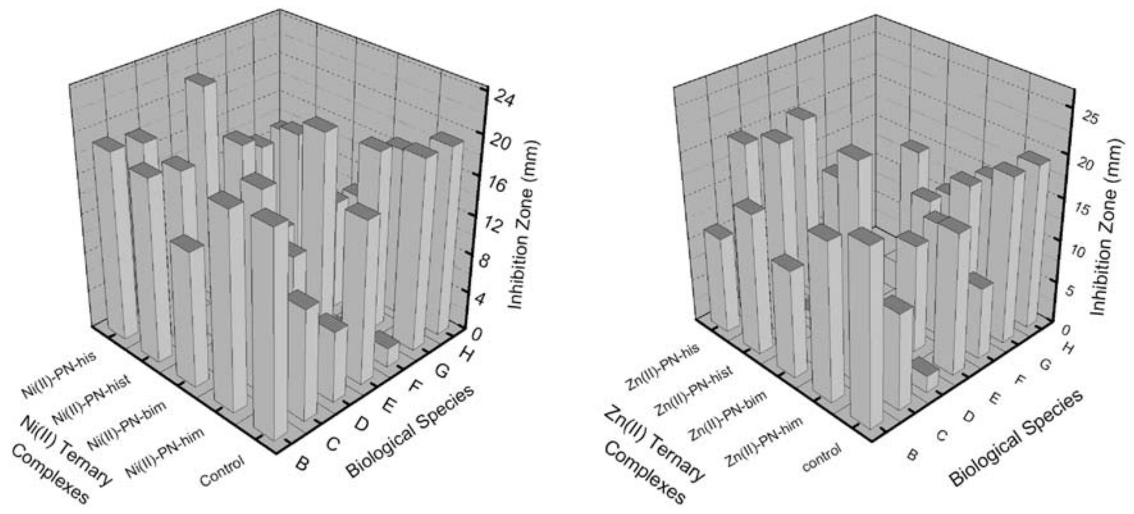


Figure 5. Biological activities of Ni(II) and Zn(II)-PN-him/bim/hist/his ternary complexes by disk diffusion method (zone formation in mm).

accounted by considering the higher basicity of him compared to that of the bim ligand.

Electronic Spectra of Ternary Complexes. The electronic spectra of Ni(II) and Cu(II) ternary complexes in aqueous solution (Figure S1 of the Supporting Information) in the pH range 4 to 7 were recorded by keeping the same experimental conditions as those for pH metric measurements. The electronic spectral parameters are given in Table 4. All of the Cu(II) complexes except Cu(II)-PN-his show a d−d envelope in the region (755 to 829) nm at pH 6.5, which is assigned to the d−d transition. This indicates that the copper complexes are in a compressed or flattened tetrahedral structure.[36] Tetrahedral copper complexes are known to be present in some metalloenzymes and metalloproteins.[37] However, the spectra of the Cu(II)-PN-his complex showed absorption at 690 nm due to the $^2E_g \rightarrow ^2T_{2g}$ transition. This indicates that the CuAB species in the Cu(II)-PN-his complex possesses distorted octahedral geometry[38] due to the tridentate binding of his and bidentate binding of PN with Cu(II) and the occupation of a solvent molecule in the remaining position. When the pH is lowered, the absorption maximum value is shifted to higher wavelength, and at pH 5.5 the absorption maximum is at 782 nm which is assignable to the d−d envelope and possesses tetrahedral geometry due to the bidentate binding of his with Cu(II) where amino group is being protonated. Hence, it can be concluded that at lower pH the Cu(II)-PN-his system possesses tetrahedral geometry, and at higher pH the geometry is octahedral. The molar conductance of the Cu(II)-PN-his solution is measured at different pH values. At lower pH, the molar conductance value of the Cu(II)-PN-his complex is ~90, and at pH 6.5 it is 10 $\text{mho}\cdot\text{cm}^{-1}\cdot\text{mol}^{-1}$, which confirms the protonation of amino group of his at lower pH values.

The electronic spectra of Ni(II)-PN-him/bim/hist/his complexes showed three d−d bands; ~ 400, 650 to 732, and ~1100 nm, which correspond to $^3A_{2g} \rightarrow {}^3T_1(P)$, $^3A_{2g} \rightarrow {}^3T_{1g}(F)$, and $^3A_{2g}\,(F) \rightarrow {}^3T_{2g}(F)$ transitions.[36] The band at (650 to 720) nm in these complexes appears as doublet, which may be due to the extensive mixing of ^1E_g and $^3T_{1g}$ states giving rise to octahedral geometry for these complexes. The nephelauxetic ratio (β) values calculated for all of the nickel complexes (binary and ternary) lie in the range of 0.67 to 0.87, suggesting covalent character of the metal ligand bond. Also, the ratio ν_3/ν_2 is ~1.60 as expected for the octahedral complexes.

Cyclic Voltammetry. The cyclic voltammetric behavior of PN and Cu(II)-PN-him/bim/hist/his complexes ($3\cdot10^{-3}$ M) were studied at glassy carbon (GC), Pt, and Ag electrodes in DMSO in the presence of 0.05 M $[\text{Bu}_4\text{N}]\text{ClO}_4$ solution as a supporting electrolyte with a scan rate of (25 to 100) $\text{mV}\cdot\text{s}^{-1}$. The electrochemical oxidation and reduction potentials were measured with respect to Ag/AgCl/KCl (satur.) [Table S1 of the Supporting Information]. Figure 4 shows the voltammogram of the ligand PN(A), Cu(II)-PN(A), and Cu(II)-PN(A)-him/bim/hist/his(B) complexes at different scan rates. In the scan rate of 100 $\text{mV}\cdot\text{s}^{-1}$ PN displays a cathodic (−0.907 V) and an anodic (0.524 V) peak. When the scan rate increases from (25 to 100) $\text{mV}\cdot\text{s}^{-1}$, the Ip_a/Ip_c value approaches one, which indicates the chemical reversibility of the process. The anodic and cathodic peaks of the free ligand (PN) disappear in the case of voltammograms of all the copper complexes indicates that PN is involved in coordination with the Cu(II) ion. All of the copper complexes have shown well-defined waves in cathodic and anodic regions. The cyclic voltammograms of Cu(II)-PN(A) and Cu(II)-PN(A)-imidazole containing ligand (B) complexes are quite different, and the cathodic peaks are slightly shifted to more negative potentials indicating the formation of ternary complexes in solution. Comparing the values of the reduction potentials of the Cu(II)-PN(A)-him/bim/hist/his(B) systems, it is clear that the stability of complexes follows the order:

$$\begin{aligned}&\text{Cu(II)-PN-his}[(E_{\text{red}}) = 212\ \text{mV}] > \text{Cu(II)-PN-hist}[(E_{\text{red}})\\&\quad = 205\ \text{mV}] > \text{Cu(II)-PN-him}[(E_{\text{red}})\\&\quad = 136\ \text{mV}] > \text{Cu(II)-PN-bim(B)}[(E_{\text{red}}) = 128\ \text{mV}]\end{aligned}$$

This order is in accordance with the results obtained by pH metric titrations under the same conditions as shown in Table 3.

Biological Activities. The antimicrobial activities of the ternary complexes (M:A:B = 1:1:1; 3.10^{-3} mM) in vitro were tested against microbes by modified disk diffusion method.[30] It has been suggested that ligands with the N and O donor system might have inhibited enzyme production, since enzymes which require free hydroxyl groups for their activity appear to be especially susceptible to deactivation by the ions of the complexes.[39] The complexes facilitate their diffusion through the lipid layer of spore membranes to the site of action and ultimately killing them by combining with −OH groups of certain cell enzymes. The variation in the effectiveness of different microbial agents against different organisms depends on the impermeability of the cell. Chelation reduces the polarity of the central metal atom, mainly because of partial sharing of its positive charge with the ligand. Also, the normal cell process may be affected by the formation of hydrogen bond through the nitrogen atom of the ligand with the active centers of cell constituents. From the results, it is clear that Ni(II) and Zn(II) complexes exhibit inhibition toward all of the studied microorganisms (Figure 5). However, Cu(II) complexes exhibit less inhibition toward the microorganisms (Table S2 of the Supporting Information).

■ CONCLUSIONS

The present study identified two dissociable protons in pyridoxine. In the metal complexes, pyridoxine is coordinated to the metal ion in a bidentate manner through the phenolate oxygen and the adjacent hydroxymethyl oxygen atoms. The incorporation of imidazole containing ligands increases the stability of the complexes. The stability of binary and ternary complexes follows the Irving−Williams series of order of metal ions. This study helped in understanding the mode of coordination of pyridoxine with metal ions in the presence of imidazole-containing ligands. The stability of ternary complexes obtained using the pH metric method is followed in the cyclic voltammetric studies also. The antimicrobial studies reveal that Ni(II) and Zn(II) complexes show an increased biological activity compared to the free ligands.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. Tables S1 and S2: cyclic voltammetric data of Cu(II) ternary complexes and biological activities of the ternary complexes, respectively. Electronic absorption spectra of ternary complexes at different pH intervals are given in Figure S1. This material is available free of charge via the Internet at http://pubs.acs.org.

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