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COMPLEXES OF VITAMIN B_6—PART XXII.* THE INTERACTION OF VITAMIN B_6 COMPOUNDS WITH CU^{II}–GLYCINEHYDROXAMIC ACID BINARY SYSTEM

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Abstract—Binary complexes of the glycinehydroxamic acid (GX) with copper(II) (GX–Cu^{II}), as well as their interaction with vitamin B_6 compounds [pyridoxol (P), pyridoxal (PL) and pyridoxamine (PM)] were studied by pH-metric, differential pulse polarography (DPP), and electron paramagnetic resonance techniques at the ionic strength of 0.15 M NaCl and at 25°C. The formation constants of the binary and ternary species were determined using the SUPERQUAD program. The three ternary systems do not show the same stoichiometries of the different species, in addition, PL interacts with GX to form Schiff bases at $pH > 4.5$. Comparative studies of the DPP of the binary and ternary systems of Cu^{II} revealed that Cu^{II} is more stabilized toward reduction in the presence of PM than in presence of GX at $pH > 6.0$; in addition the redox behaviour of Cu^{II} in the presence of PM is similar to that of the GX–PM–Cu^{II} ternary system. On the other hand, the DPP of the ternary systems GX–P (or PL)–Cu^{II} are similar to those of GX–Cu^{II} binary system at different pH values. The nuclear spin-electron spin coupling of Cu^{II} in these systems is discussed.

In recent years, the chemistry of aminohydroxamic acids has received considerable attention, probably due to the importance of their metal complexes in biological systems.[1–3] Apart from their usefulness in chemical analysis, they have been employed in many biological aspects such as the treatment of the diseases urolithiasis[4] and hepatic coma.[5] Besides this, they have been tested also as tumour inhibitors[3] and they have been used in attempts to design metal chelates as suitable oral sources of metals for mammals.[6]

In fact, aminohydroxamic acids form quite stable binary complexes with many transition metal ions. Their equilibria and mechanism of complex formation in solutions have been the subject of several reports.[7–12] Similarly, vitamin B_6 compounds, VB_6 [pyridoxol (P), pyridoxal (PL) and pyridoxamine (PM)] and their binary metal complexes were also the subject of many research papers for many years especially those involving pyridoxal and pyridoxal-5′-phosphate.[13–18] Apart from their biological function, they have been described for the treatment of carpal tunnel syndrome,[19] enhancing hair growth and ophthalmological disorders.[20]

The literature on ternary metal complexes involving aminohydroxamic acids and vitamin B_6 compounds is very sparse.[21] Interest in these ternary complexes has been initiated recently in our laboratory because of their possible application in chemotherapy by combining the therapeutic properties of the aminohydroxamic acid and vitamin B_6 compounds in one compound and in the modelling of metal ion transfer between low molecular weight complexes and biological macromolecules.

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EXPERIMENTAL

Materials

Pyridoxol hydrochloride (P.HCl), pyridoxal hydrochloride (PL.HCl) and pyridoxamine dihydrochloride (PM.2HCl, Merck ~99%, Fig. 1a) were analytically pure chemicals and were used without purification. Stock solutions of 0.10 M of the vitamin B_6 compounds were prepared by the accurate weighing of their appropriate amounts and dissolving in doubly distilled water. The solutions were kept in the dark at ~4°C. Stock solution of glycinehydroxamic acid (Fig. 1, Sigma >98%), GX, was also prepared by accurate weighing and dissolved in 0.10 M HCl. Stock aqueous solution of $CuCl_2$ (BDH) of ~0.205 M was checked for concentration by EDTA compleximetric titration using potentiometric methods as previously described.[17] Ionic strength was kept constant at 0.15 M NaCl. Carbonate free NaOH of the concentration 0.0972 or 0.1005 M prepared from J. Baker NaOH ampoules was used in the pH-metric titration.

Measurements

Potentiometric titrations were performed using a Metrohm 670 titroprocessor equipped with Metrohm 6.0102.100 glass and 6.0702.100 calomel (containing 3 M KCl) electrodes. The electrode system was calibrated before use with three Schott Gerate buffers of 4.01, 6.87 and 9.18 pH values. Titrant was delivered by the Metrohm 665 Dosimat accurate to ±0.001 ml. The titration cell was thermostated at 25°C. The sample solutions were purged by humidified ultra pure nitrogen. The pH-metric titration of the binary system of Cu^{II}–GX was done using a combination of Orion Research Ionalyzer type 901 provided with Orion (91-01) glass and (90-05-00) calomel electrodes, calibrated with only two buffers of 4.01 and 6.87, Schott Gerate autoburette type TA50 SGH accurate to ±0.01 ml. The titrations were done under the same experimental conditions of the Metrohm settings. The concentration ranges of the metal ions and ligands are listed in Table 1.

The EPR solution spectra (at different pH values) were obtained at room temperature using an X-

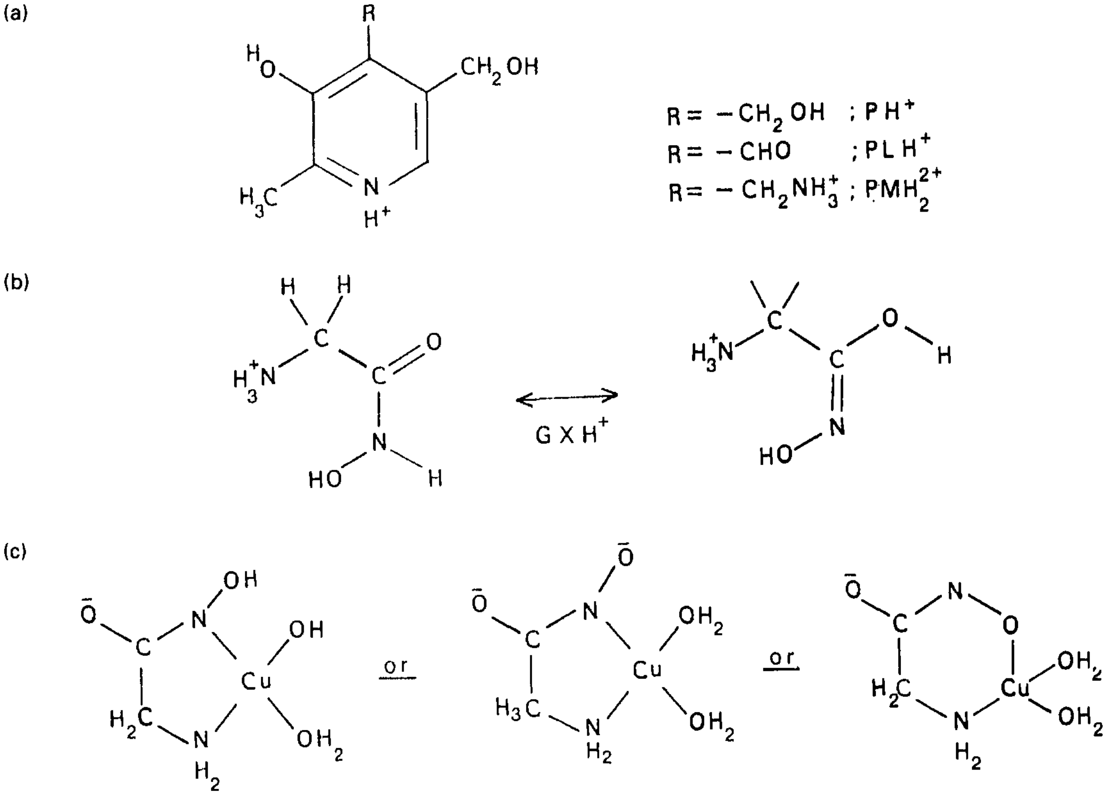

Fig. 1. Schematic diagram of the structural fomulae of: (a) vitamin B_6 compounds; (b) glycinehydroxamic acid; (c) GX–Cu^{II} hydroxo species (1:0:1:–1); (d) GX–Cu^{II} trimer species (4:0:3:0); (e) GX–VB_6–Cu^{II} species (3:1:3:0); (f) GX–PL–Cu^{II} Schiff's base.

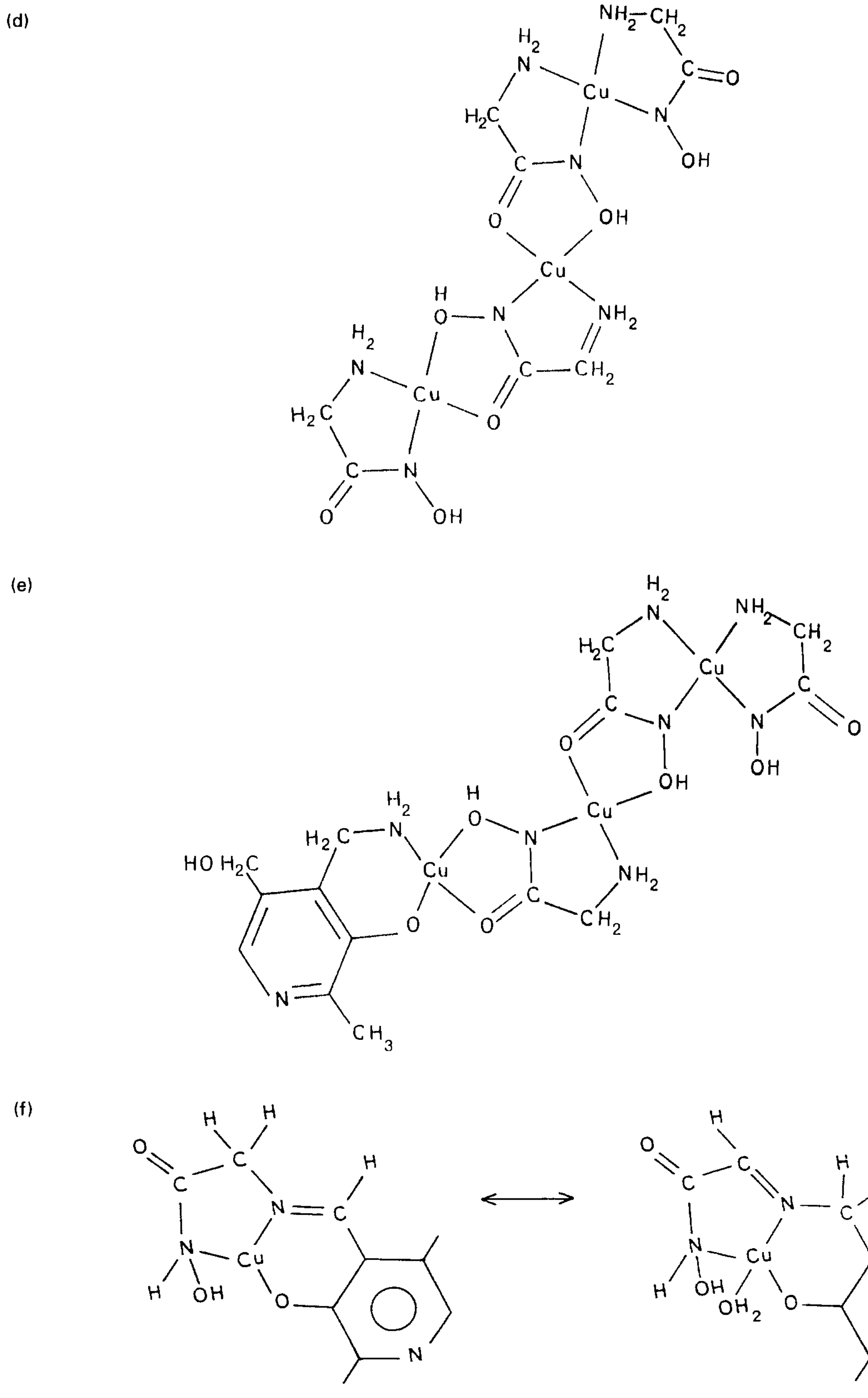

Fig. 1. *Continued.*

Table 1. Summary of the experimental conditions encountered in titrimetric analysis

System	$T_{GX} \times 10^3$ M	$T_P \times 10^3$ M	$T_{PL} \times 10^3$ M	$T_{PM} \times 10^3$ M	$T_{Cu} \times 10^3$ M	pH range
GX–Cu^{II}	1.0–6.0	—	—	—	1.0–2.0	3.00–11.0
GX–P–Cu^{II}	—	1.0–6.0			1.0–2.0	3.20–10.5
GX–PL–Cu^{II}			1.0–6.0		1.0–2.0	2.50–11.0
GX–PM–Cu^{II}				1.0–6.0	1.0–2.0	2.20–11.0

band (9.85 GHz) Bruker ECS 106 EPR spectrometer operating at 100 kHz modulated frequency with modulated amplitude of 10.115 G in the magnetic field range 2700–3700 G. The sample concentrations were: 5.0×10^{-3} M Cu^{II} ions, 1.0×10^{-3} M GX, 1.0×10^{-3} M P, 1.0×10^{-3} M PL and 1.0×10^{-3} M PM. The pH of the solutions were adjusted by Na_2CO_3. The ionic strength was adjusted to 0.15 M by the appropriate addition of NaCl amount (2 M stock solution).

The differential pulse polarograms (DPP) were obtained by using Metrohm Polarocord E-506 provided with a three-electrode arrangement; a dropping mercury indicator electrode, a saturated calomel reference electrode and a counter platinum-wire electrode. The settings of the polarograph were as follows: the voltage range was either 0.00 to −0.50 or +0.20 to −0.50 V, pulse amplitude was −40.0 mV, drop time was 1.0 s, the recorder speed was 0.50 mm s^{-1}. The pH range used was ~3.0–12.0. Moreover, the solution was deoxygenated by passing pure humidified nitrogen before taking the polarograms. The surface of the solution was also kept under the same N_2 flow during the experiments. The ionic strength was adjusted to 0.15 M Cl$^-$. The concentration of the ligands was either 10 times or twice that of total metal ion concentration, T_{Cu}. The polarograms were taken at room temperature (23 ± 1°C).

Calculations

The formation constants of the binary species of Cu^{II}–GX system and the ternary species of the Cu^{II}–GX–VB$_6$ system were determined by using the SUPERQUAD[22] program. The basis of adopting the equilibrium model was as previously reported.[8] In this work an allowance for error in pH and the volume of titrant has been made in the range 0.001–0.005 pH unit and 0.005–0.0010 cm^3, respectively.

RESULTS AND DISCUSSION

The potentiometric study

(i) *Binary complexes of the Cu^{II}–GX system.* Titrimetric analysis of solutions of different molar ratios of the ligand to metal, Table 1, indicated that the complex formation started at pH ≈ 3.0. Furthermore, no precipitation was observed when the molar ratios of the ligand to the metal ion was ⩾2 : 1 over the pH range 3.0–11.0. However, precipitation was observed when the molar ratios of the ligand to the metal ion were 1 : 1 at pH ⩾ 8.0. The colour change of the solution from initially almost colourless to light green–violet accompanied the change in the pH in the range ~3.0–6.0.

The assessment of the titration data with SUPERQUAD program has produced two equilibrium models (Table 2). The species of the first model resembles those which was previously reported,[10] except that an extra species has been found in this work (4 : 0 : 3 : 1 for GX : VB$_6$: Cu^{II} : H$^+$). The species of the second model, on the other hand, also has an extra species of 1 : 0 : 1 : −1. Although in both cases the dimeric (2 : 0 : 2 : −1) species was confirmed to exist, yet the percentage amount did not exceed 40% of the total metal ion concentration, T_{Cu}, in the pH range ~4.0–6.4 (Fig. 2) which is in contradiction to the previously reported percentage value (~80%).[10] This may be due to the difference in experimental conditions where the ionic strength used[10] was 0.10 M NaClO$_4$ whereas in the current work it is 0.15 M Cl$^-$.

The structures of the complexes 1 : 0 : 1 : 0, 2 : 0 : 1 : 0, 2 : 0 : 1 : −1 and 2 : 0 : 2 : −1 species have been previously suggested on the basis of absorption and EPR spectra.[10] The structure of the newly obtained species 1 : 0 : 1 : −1 may similarly be predicted as shown in Fig. 1(c) in which either a hydrolysed (hydroxo) species has been formed, which actually occurred in many binary metal complexes of Cu^{II} when there is deficiency in ligand concentration of coordinate Cu^{II}[23] where deprotonation of one of the inner coordinating water molecules is facilitated, or the N—OH proton of the hydroxamate group is ionized coordinating through the N of amino group and the hydroxamate group or through the N or amino group and the oxygen of hydroxamate group. In addition, the structure of the 4 : 0 : 3 : 0 species which exist in the first equilibrium model may be visualized as if

Table 2. Summary of the protonation constants of the ligands and the formation constants of their binary metal complexes of Cu^{II} with GX, PL, P and PM

System	Stoichiometry				log $\beta(\pm\sigma)$	pH range	Reported log β
	l	p	q	s			
GX–H	1	0	0	1			9.55[a]
	1	0	0	2			17.15[a]
PL–H	0	1	0	1			8.48[a]
	0	1	0	2			12.57[a]
P–H	0	1	0	1			9.00[b]
	0	1	0	2			13.95[b]
PM–H	0	1	0	1			10.41[c]
	0	1	0	2			18.56[c]
	0	1	0	3			22.06[c]
GL–PL–H	1	1	0	0			4.34[a]
	1	1	0	1			13.04[a]
	1	1	0	2			21.04[a]
PM–Cu^{II}–H[d]	0	1	1	0			10.81
	0	1	1	1			17.22
	0	1	1	2			21.34
	0	2	1	3			38.91
	0	2	1	0			17.47
	0	2	1	1			25.46
	0	2	1	2			32.54
P–Cu^{II}–H	0	1	1	1			11.60[e]
	0	2	1	2			18.41[b]
PL–Cu^{II}–H[f]	0	1	1	0			3.51
	0	2	1	0			7.00
Model 1							
GX–Cu^{II}–H	1	0	1	0	11.53(0.04)	3.30–11.0	10.83[g]
	2	0	1	0	21.19(0.05)		19.89[g]
	2	0	1	−1	11.56(0.12)		9.95[g]
	2	0	2	−1	21.41(0.06)		20.91[g]
	4	0	3	0	51.80(0.12)		
Model 2							
GX–Cu^{II}–H	1	0	1	0	11.11(0.06)	3.70–11.0	10.83[g]
	2	0	1	0	20.95(0.03)		19.89[g]
	1	0	1	−1	6.08(0.08)		
	2	0	1	−1	11.29(0.09)		9.95[g]
	2	0	2	−1	21.73(0.03)		20.91[g]

[a] Reference 7.
[b] Reference 14.
[c] Reference 3.
[d] Reference 31.
[e] Reference 11.
[f] Reference 32.
[g] Reference 10.

the ligand makes available other coordination sites through the oxygen atoms of the N—OH and CO groups (Fig. 1d), in addition to the suggested ligating sites of the nitrogen atoms of NH_2 and NOH groups. This kind of species has been encountered in the binary system of the methionine hydroxamate Cu^{II} [24] and N-hydroxy-D-asparagine Cu^{II}.[25]

Table 2 shows also the protonation constants of the vitamin B_6 compounds together with the formation constants of their binary complexes with Cu^{II}.

(ii) *Ternary complexes of Cu^{II}—GX—VB_6 systems.* The addition of vitamin B_6 compounds, P, PL or PM, to the GX–Cu^{II} system did not show

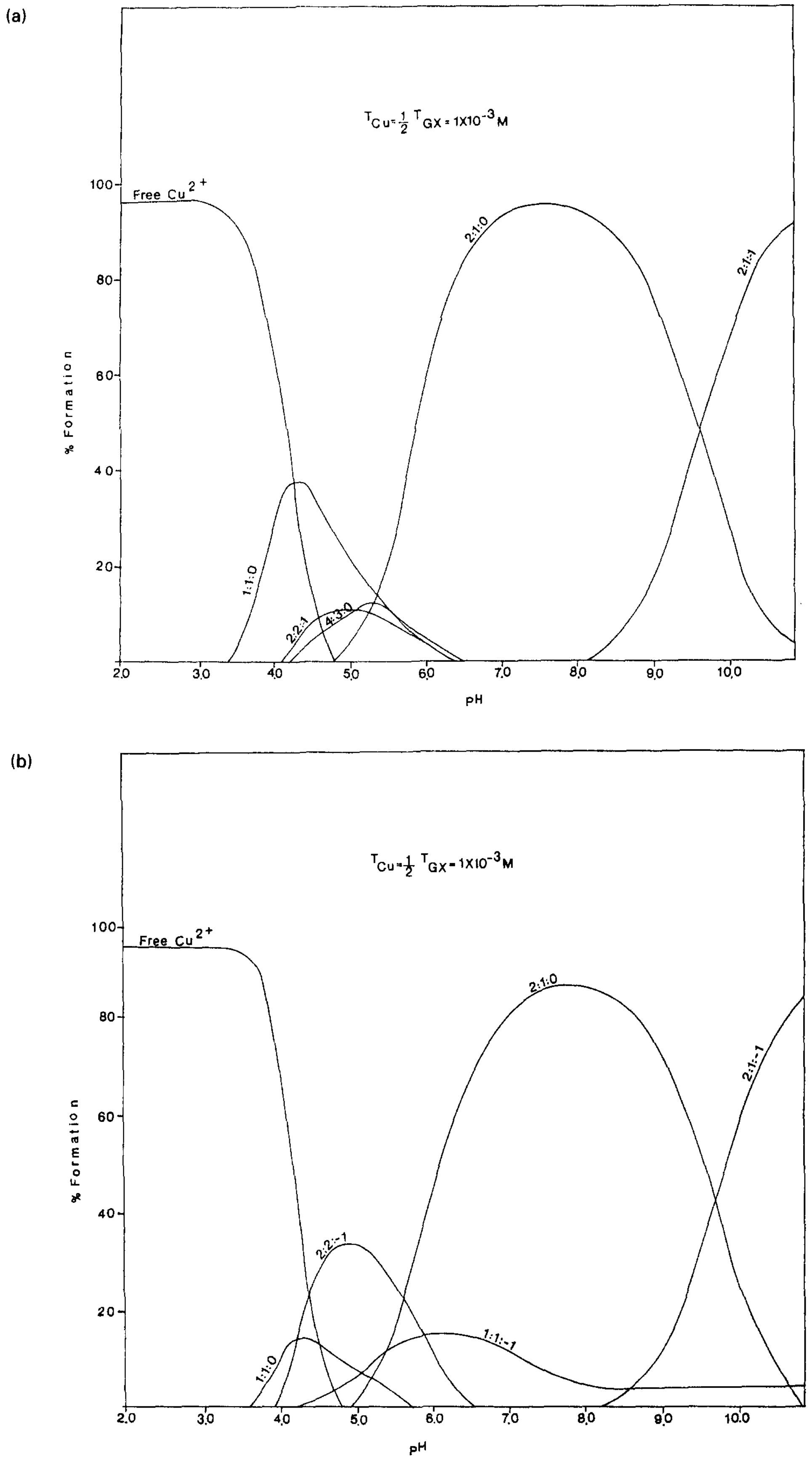

Fig. 2. The distribution curves of the species in the binary system GX–Cu^{II} as a function of pH.

any precipitation in the pH range of 3.0–11.0 for all the metal to ligand ratios in Table 1, with the exception of the 1 : 1 : 1 ratio of GX : P : Cu^{II} which slightly precipitates at pH ~8.0; however, it redissolved at higher pH values.

Many equilibrium models have been tested, using the SUPERQUAD program, to assess the titration data. Statistical criteria were only taken into consideration to prove the validity of the selected model assuming errors in pH and volumes of titrant up to 0.005 and 0.01, respectively. Table 3 depicts the formation constants and stoichiometries of different species. The three ternary systems do not, in general, show the same species stoichiometry, a conclusion which indicates variation in the ligation properties of vitamin B_6 compounds toward GX–Cu^{II} binary systems. Since GX is a multidentate ligand with three chelation sites, one should expect that it will preserve the same property in the ternary complexes. So one should conclude that vitamin B_6 compounds will act as a monodentate or bidentate ligand toward Cu^{II}. Few complex species are not protonated, 1 : 1 : 1 : 0, 3 : 1 : 3 : 0, 2 : 1 : 2 : 0 or 2 : 2 : 1 : 0 (GX : VB_6 : Cu^{II} : H) and the majority are protonated, with protons probably located on the VB_6 moieties. These findings have been partially encountered in the ternary species of bipyridyl (BP) and vitamin B_6 compound complexes with Cu^{II}.[23] Comparable protonated species in the ternary systems involving P or PL have approximately the same formation constants emphasizing the similarity between the ligation properties of both vitamin B_6 compounds. It has been previously shown that BP enhances the interaction of VB_6 compounds with Cu^{II}.[23] If it is assumed that GX ligates Cu^{II} through the nitrogen of the amino acid hydroxamate groups, comparison with BP is worthwhile despite the difference in the structures of both ligands. In general, comparable species are more stable for GX systems than BP systems. This is expected since BP exhibits relatively greater steric strain. The PM systems have almost the same species except that the GX containing ternary species has less protonated species than BP containing species. Besides, trimer species are exhibited by the former system [Fig. 1(e)], probably due to the presence of more than one effective chelation site in GX. Figure 3(a) shows the distribution curve of the ternary system; GX–PM–Cu^{II}. On the other hand P or PL–GX ternary systems are quite different from those of P or PL–BP systems. In the case of the GX–P–Cu^{II} system, more than one molecule of GX and P is involved in some of the species (Table 3) while this situation was not found in the BP–P–Cu^{II} system. This was attributed to less repulsion between the ligands in the former systems. In such a case one should expect that P ligate through the pyridine nitrogen rather than through the phenoxy

Table 3. Summary of the ternary complex formation constants obtained from SUPERQUAD for the systems GX–VB_6–Cu^{II}–H at 37°C and $I = 0.15$ M NaCl. p, q, r, s are the stoichiometric coefficients standing for GX–VB_6–Cu^{II}–H, s the standard deviation in data, χ^2 the chi square and n the no. of titration points

	Stoichiometric coefficients					
System	p	q	r	s	log $\beta(\pm\sigma)$	pH range, s, χ^2 and n used
GX–PM–Cu^{II}–II	1	1	1	0	20.09(0.05)	3.2–10.4, 1.32, 9.7 and 502
	1	1	1	1	27.78(0.04)	
	2	2	1	2	47.29(0.05)	
	3	1	3	0	52.64(0.15)	
GX–P–Cu^{II}–H	1	1	1	1	23.74(0.06)	3.7–9.0, 1.53, 6.32 and 350
	1	2	1	1	28.58(0.10)	
	1	2	1	2	35.96(0.05)	
	2	2	1	1	36.28(0.10)	
	2	2	1	2	44.96(0.06)	
GX–PL–Cu^{II}–H[a]	1	1	1	3	31.40(0.02)	3.4–8.9, 0.73, 24.5 and 516
	2	2	1	1	35.72(0.09)	
	2	1	2	0	33.28(0.03)	
	1	2	1	1	28.16(0.03)	
	2	1	1	1	32.37(0.04)	
	2	2	1	0	27.01(0.14)	

[a] In the calculation of the formation constants of the ternary species of this system protonation constants of the ligands, their binary formation constants and Schiff base complexes were kept constant while varying those of the ternary species.

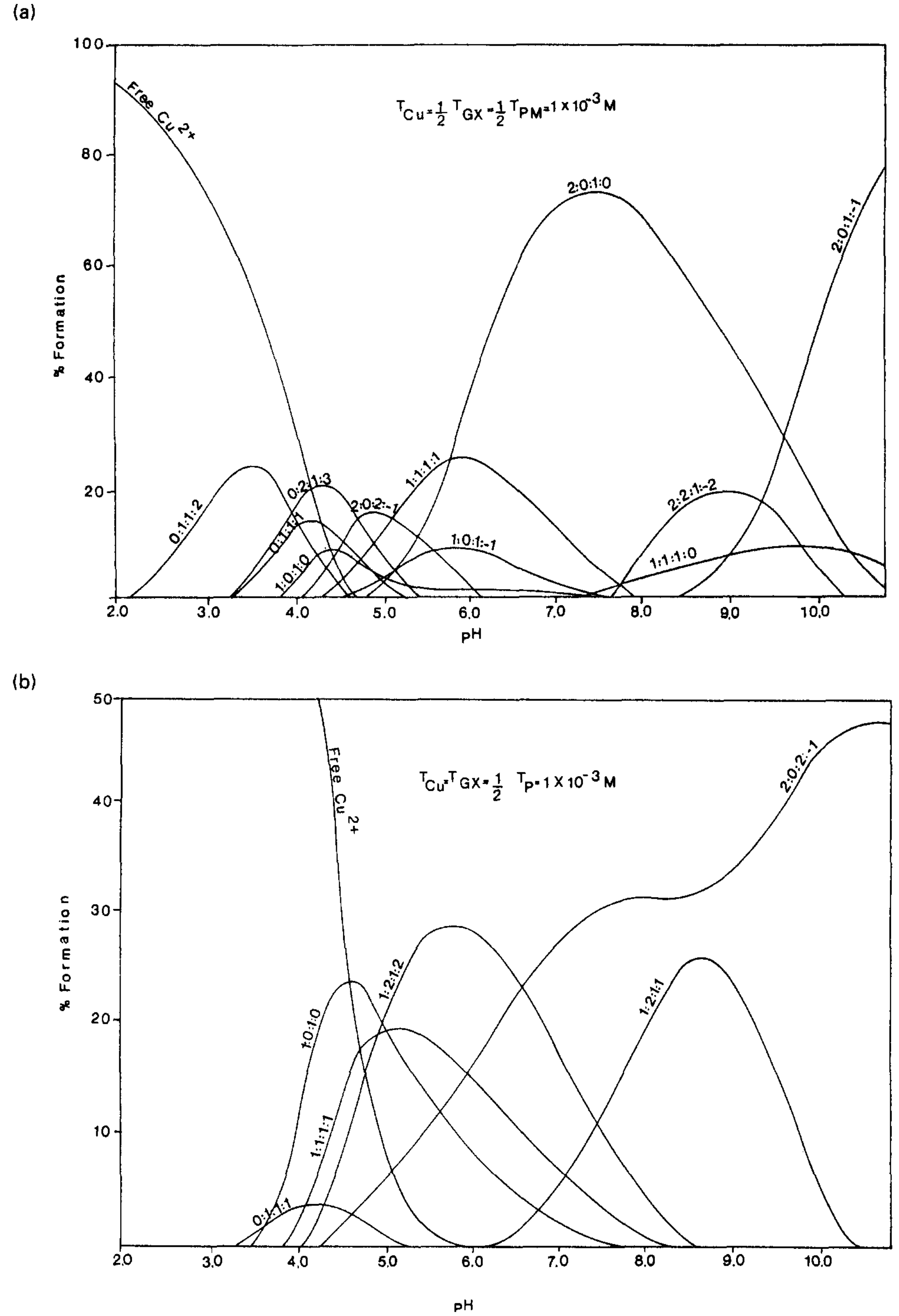

Fig. 3(a) and (b).

oxygen, while GX is assumed as the bidentate nature in ligation with $\mathrm{Cu^{II}}$. Figure 3(b) shows the distribution curve of the ternary system GX–P–$\mathrm{Cu^{II}}$. In addition, it has been reported that PL interacts with GX to form Schiff bases[7] (Table 2) at pH > 6.7. However, in the presence of $\mathrm{Cu^{II}}$ this kind of interaction between GX and PL may be enhanced at pH < 6.7. In such a case a change in pH with time may be observed at pH > 4.0 similar to what has been reported with respect to the interaction of PL or PLP (pyridoxalphosphate) with PM or PMP (pyridoxamine phosphate).[26] However, no appreciable change in pH with time was observed experimentally. The equilibrium study of a GX–PL–$\mathrm{Cu^{II}}$ system cannot confirm if the ligands are of the condensed type (Schiff base) or

(c)

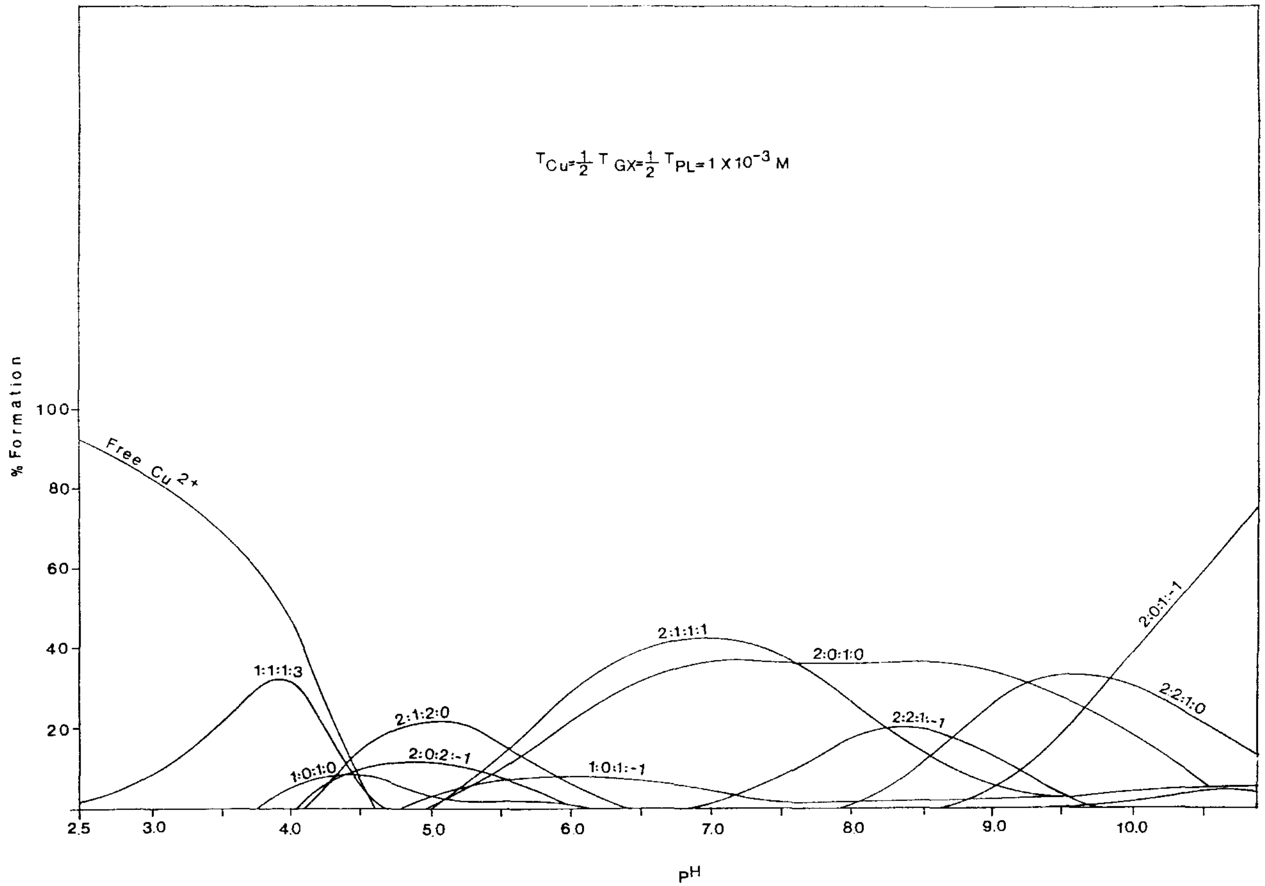

Fig. 3. The distribution curves of the species in the ternary systems: (a) GX–PM–Cu^{II}; (b) GX–P–Cu^{II}; (c) GX–PL–Cu^{II} as a function of pH.

non-condensed type. The condensed ligand should have a total of three dissociable protons while the non-condensed species has four. Since the equilibrium model includes the species 1 : 1 : 1 : 3 (GX : PL : Cu^{II} : H) one should rule out the formation of the condensed species at pH < 4.5. However, other species do not rule out the involvement of Schiff base ligand, especially at pH > 6.7.

The ternary enhancement[27] may be determined by calculating the log K values of the formation of the ternary complexes from the parent binary complexes. Table 4 details some of the values calculated whenever the parent binary species formation constants are available. The negative values of log $K_1 (< -0.6)$[28,29] indicate that the second ligand does not prefer to bond to the binary complexes rather than to the solvated metal ion. On the other hand, values $> +0.6$ for log K_2 suggest the stabilization of the ternary complex. The negative value of log K_5 (Table 4) indicates that the ternary complex cannot be formed from the binary species; however, the distribution curve [Fig. 3(c)] shows considerable formation of this species. Actually, this is not a ternary complex, it is a binary species where the ligands are now the condensed GX and PL (Schiff base). In such a case, Cu^{II} may assume six coordination.

Polarographic study

(i) *Binary systems.* The DPPs of $CuCl_2$ solution at pH $\leqslant 5$ have two cathodic peaks at ~ -64 and ~ -220 mV which have been attributed to the stepwise reduction of Cu^{2+} in the presence of Cl^-.[23] The same behaviour was exhibited by the GX–Cu^{II} system with the exception that it was extended to a wider pH range with the evolution of an extra peak (E_{3GX}) at pH values >5.0 ($\simeq 340$ mV). The two former E_{max} terms, E_{1GX} and E_{2GX}, shift slightly to more negative potentials as the pH was increased. E_{3GX} decreases in value in the pH range 4.5–6.0 and it was almost constant in the pH range ~ 6.0–9.5 (Fig. 4). A further shift to more negative potential was observed for E_{3GX} as the pH was increased at pH >9.5 (Fig. 4). The behaviour at pH $\leqslant 6.0$ was probably due to the formation of 1 : 0 : 1 : 0

Table 4. Summary of log K_i described in this work

Stoichiometry				log K	GX–P–Cu^{II}	GX–PL–Cu^{II}	GX–PM–Cu^{II}
1	1	1	0	log K_1	—	—	−1.55
1	1	1	0	log K_2	—	—	+1.52
1	1	1	1	log K_1	+0.61	—	−0.97
1		1	1	log K_2	+7.88	—	+1.83
1	2	1	1	log K_3	−0.66	—	—
				log K_4	−4.58	—	—
2	2	1	0	log K_5	—	−1.18	—
2	2	1	1	log K_6	−3.34	—	—
2	2	1	2	log K_7	+5.36	—	+3.56
2	1	2	0	log K_8	—	+8.58	—

K_1, K_2, K_3, K_4, K_5, K_6, K_7 and K_8 are for the following equilibrium reactions (charges are omitted).

$$CuGX + Cu(VB_6)H_i \rightleftharpoons CuGX(VB_6)H_i + Cu \qquad K_1 = \beta_{111i}/\beta_{1010}\beta_{011i}$$

$$Cu(GX)_2 + Cu(VB_6)H_i \rightleftharpoons 2CuGX(VB_6)H_i \qquad K_2 = \beta_{111i}/\beta_{2010}\beta_{021i}$$

$$CuGX + Cu(VB_6)_2H_2 \rightleftharpoons CuGX(VB_6)_2H + Cu + H \qquad K_3 = \beta_{1211}/\beta_{1010}\beta_{0121}$$

$$Cu(GX)_2 + 2Cu(VB_6)H \rightleftharpoons CuGX(VB_6)_2H + CuGX + Cu + H \qquad K_4 = \beta_{1211}\beta_{1010}/\beta_{2010}\beta^2_{0111}$$

$$Cu(GX)_2 + Cu(VB_6)_2 \rightleftharpoons Cu(GX)_2(VB_6)_2 + Cu \qquad K_5 = \beta_{2210}/\beta_{2010}\beta_{0210}$$

$$Cu(GX)_2 + Cu(VB_6)_2H_2 \rightleftharpoons Cu(GX)_2(VB_6)_2H + Cu + H \qquad K_6 = \beta_{2211}/\beta_{2010}\beta_{0212}$$

$$Cu(GX)_2(VB_6)_2H_2 + Cu \rightleftharpoons Cu(GX)_2(VB_6)_2H_2 + Cu \qquad K_7 = \beta_{2212}/\beta_{2010}\beta_{0212}$$

$$Cu(GX)_2 + Cu(VB_6) \rightleftharpoons Cu(GX)_2(VB_6) + Cu \qquad K_8 = \beta_{2120}/\beta_{2010}\beta_{0110}$$

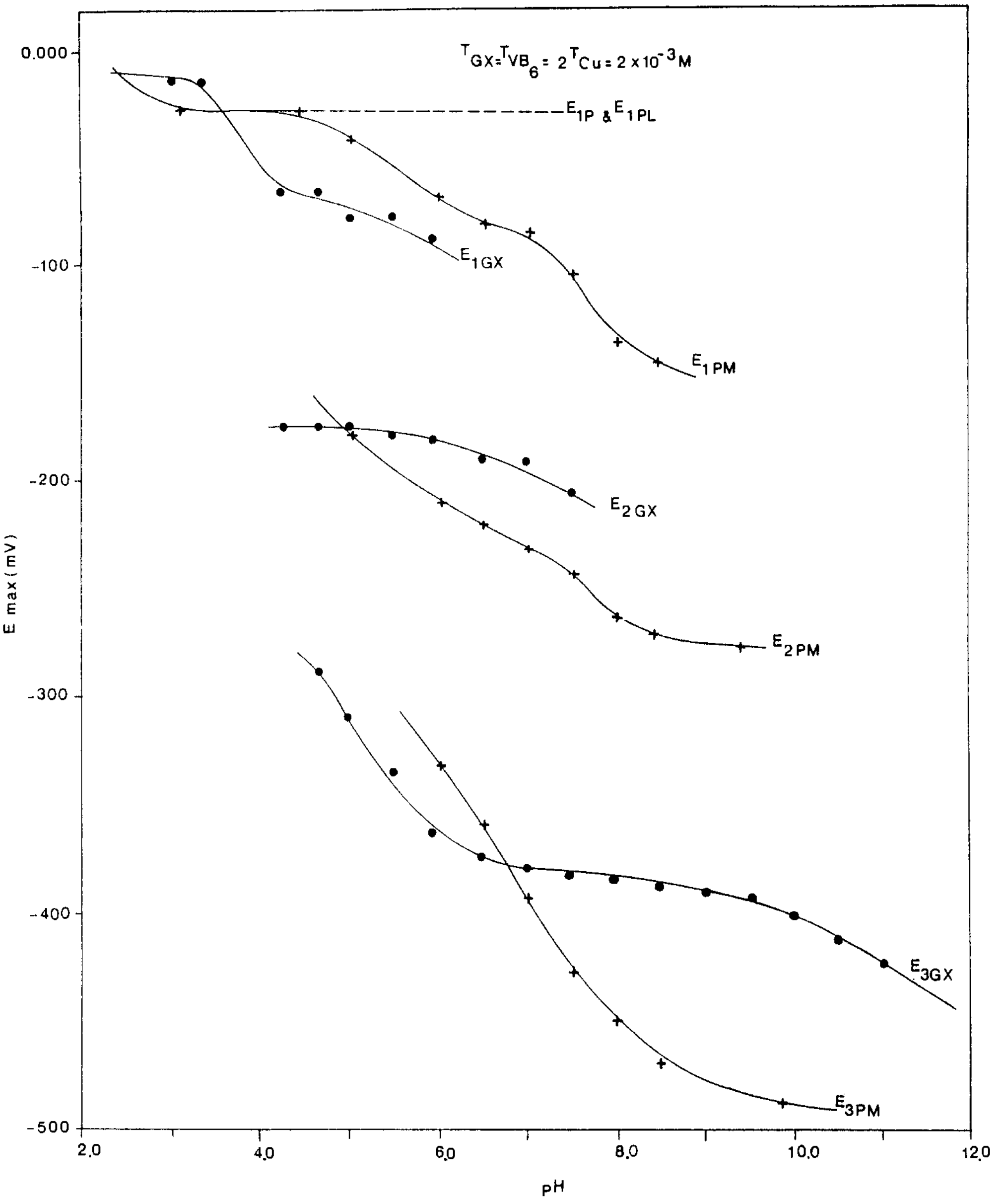

Fig. 4. The variation of E_{max} with pH for GX–Cu^{II} and VB_6–Cu^{II} systems.

(GX : VB_6 : Cu^{II} : H) species in addition to the formation of the polymeric species (i.e 2 : 0 : 2 : −1 and/or 4 : 0 : 3 : 0, Fig. 2). It is not unreasonable to assume that E_{3GX} corresponds to the formation of a highly stable polymeric species while E_{1GX} and E_{2GX} correspond to the stepwise reduction of 1 : 0 : 1 : 0 at pH < 6.0. Certainly, the constant value of E_{3GX} in the pH range ~6.0–9.5 (Fig. 4) should correspond to the formation of a 2 : 0 : 1 : 0 species (Fig. 2) and the values of E_{3GX} in the pH range >9.5 should correspond to the reduction property of the species 2 : 0 : 1 : −1. The half peak width varies between > 100 and 52 mV, being close to 52 mV at pH > 10.0 (i.e. a two electron reduction process). This conclusion indicates that Cu^{II} is well stabilized toward reduction in the presence of GX and stepwise reduction occurs at pH < 10.0.

The reduction properties of Cu^{II} in the presence of PM are similar to those with GX–PM–Cu^{II}. It exhibits three peaks E_{1PM}, E_{2PM} and E_{3PM} which shift to more negative potentials as pH is increased (Fig. 4). However, Cu^{II} is more stabilized towards reduction in the presence of PM than in the presence of GX especially at pH > 6.0. both E_{1PM} and E_{2PM} show an inflection at pH = 7.0 which marks the stepwise transformation from 0 : 1 : 1 : sH to 0 : 2 : 1 : sH. The protonation or deprotonation of the complex species seems not to affect greatly their reduction properties.

The reduction properties of Cu^{II} in the presence of P or PL exhibit only a single peak (E_{1P} and E_{1PL}) at ~−74 mV. The pH variation (at pH < 6.0) has almost no effect on E_{1P} and E_{1PL} (Fig. 4). Although the loss of the Cu^{II} peak at ~ −220 mV indicates complexation with P or PL, at pH < 6.0 destabilization of the Cu^{II} oxidation state occurred.

(ii) *Ternary systems*. Although the DPPs of the ternary systems GX–P (or PL)–Cu(II) show much similarity to those of the GX–Cu^{II} system at different pHs, yet that of GX–PM–Cu^{II} is different in some respects. The former systems exhibit three E_{max} terms while the latter has four. Figure 5 displays the variation of E_{max} as a function of pH. In general, the pattern of the curves is different from that of the binary systems (Fig. 4). The first E_{max} in the systems (E_1) agree in having an inflection at pH ≃ 4.5 which marks the change from the formation of 1 : 1 : 1 : s [GX : P (or PL or PM) : Cu^{II} : sH] species to the formation of other ternary species (Fig. 3). In all these systems the presence of E_1 and E_2 is still preserved, especially at pH < 7.0, demonstrating the stepwise reduction of Cu^{II}. However, both peaks are shifted to higher pH (~4.0, Fig. 5). The third E_{max} (E_3) of all systems appeared at pH ~ 4.5 which corresponds to the formation of the highly coordinated Cu^{II} complex species associ-

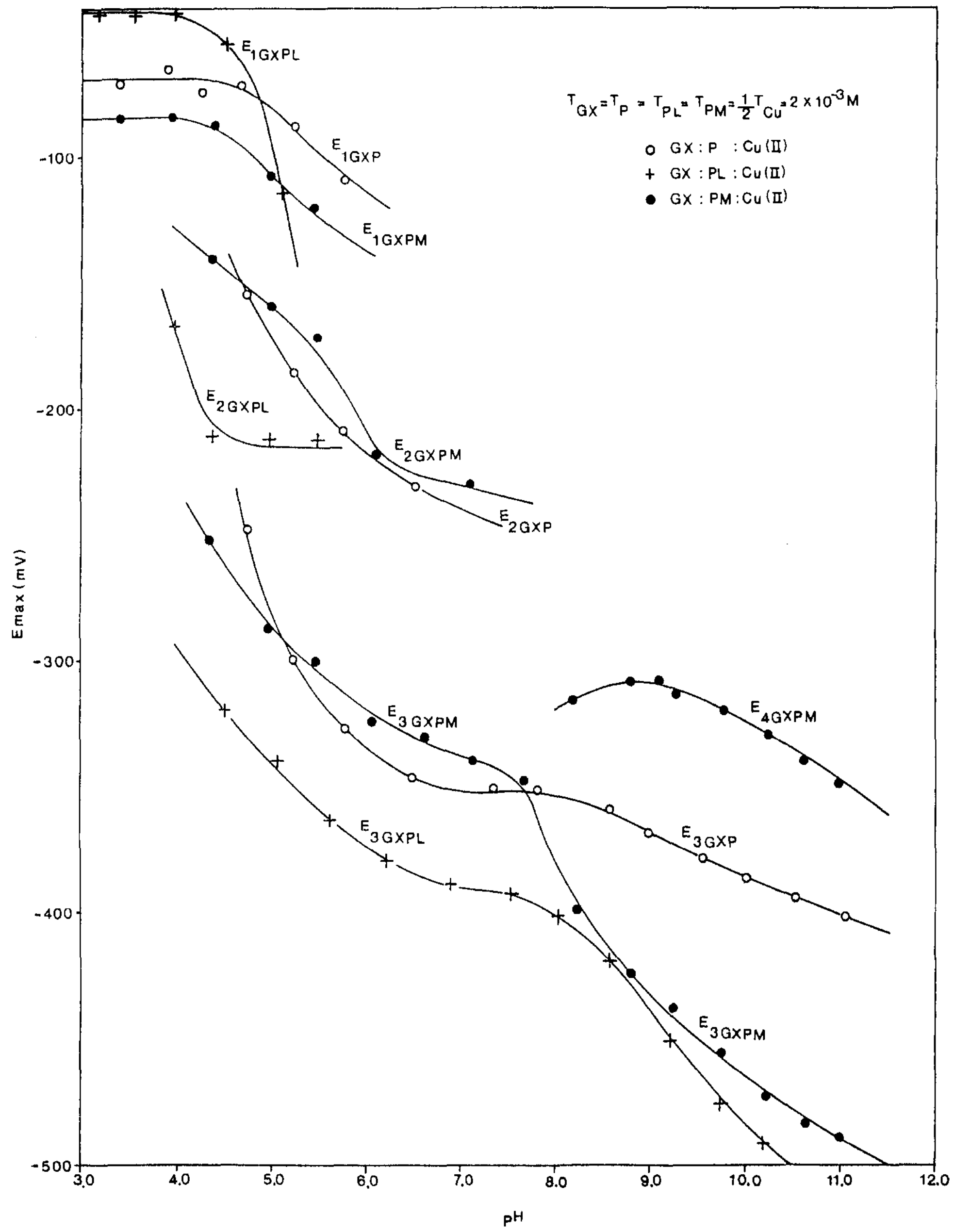

Fig. 5. The variation of E_{max} with pH for GX–VB$_6$–Cu^{II} systems.

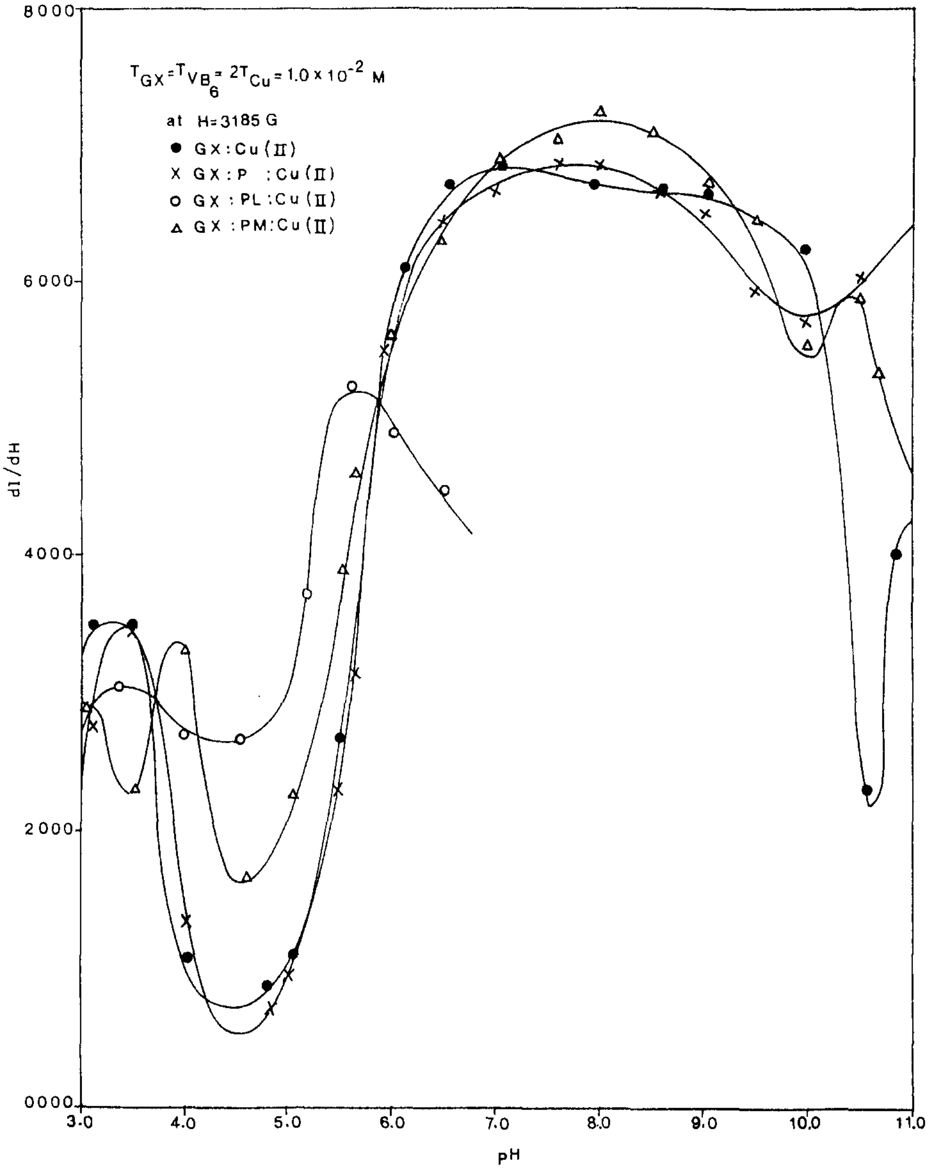

Fig. 6. The variation of dI/dH with pH for GX–Cu^{II} and GX–VB_6–Cu^{II} systems.

ated with stabilization of the oxidation state due probably to the ligand π-back bonding. The half-peak widths of E_3 at pH $\simeq 7.5$ for the ternary systems GX–P or PL or PM–Cu^{II} are ~ 88, 68 and 100 mV respectively. Assuming reversibility, the values indicate a one to two electron reduction process. The constant value of E_{3GX} of the binary system in the pH range 6.0–9.5 is also shown in the ternary systems with shorter pH range (Fig. 5), emphasizing the formation of ternary species (Fig. 3). The formation of a fourth E_{max} in the GX–PM–Cu^{II} system at higher potentials of pH > 8.0 (Fig. 5) may provide evidence of the stepwise reduction of Cu^{II} of the highly coordinated species or may correspond solely to the formation of the ternary species, 2 : 2 : 1 : 2 and 1 : 1 : 1 : 0 (Fig. 3).

The ternary systems show a peculiar behaviour at pH > 9.0, a new sharp E_{max} ($W_{1/2} \simeq 20$ mV) at potentials close to 0 mV which shifts to lower potentials as pH increased. This has been attributed to the reduction properties of ligands in the presence of Cu^{II}. They are almost absent in the free ligands, in the potential range used, except at pH > 11.0.

EPR Studies

The EPR spectra of $CuCl_2$ and $CuClO_4$ (at ~ 0.15 M ionic strength) are practically the same at pH <5.0. They consist of one signal at 3209 G indicating no nuclear spin–electron spin coupling in the presence of weakly complexing media. The g_{av} factor is 2.193. Probably the major cause of this

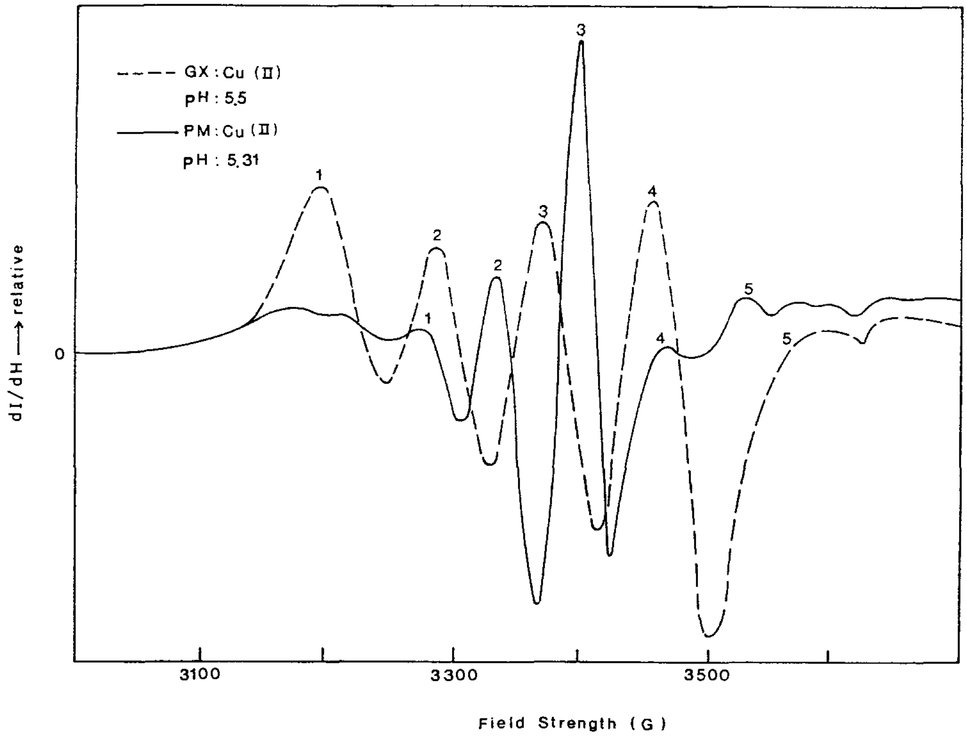

Fig. 7. The variation of dI/dH with field strength (G) for GX–Cu^{II} and PM–Cu^{II} systems.

broad line is the unresolved interaction of the unpaired electrons with the protons of water molecules coordinated to the metal ions in solution.[30]

Three phenomena have been observed upon the addition of GX to $CuCl_2$ solution at pH $>$ 3.0. Firstly, the intensity of the EPR signal of Cu^{II} decreased as the pH was increased due to complex formation. Secondly, the EPR signal was shifted to higher magnetic field, 3352 G (clearly observed at pH $>$ 4.8 where the g_{av} factor is now equal to 2.100. Thirdly, hyperfine splitting due to nuclear spin–electron spin coupling resulted in the formation of 2–4 signals as the pH was increased, two signals in the pH range 3.0–4.0 and four signals at pH $>$ 4.0. The typical coupling constant is ~86 G at pH $>$ 5.0. However, in a narrow pH range of 4.4–5.0, the signals almost vanished. This has been attributed to the formation of dinuclear and/or polynuclear species where possibly the antiferromagnetism played a role in this phenomena, Figs 2 and 6. At pH $>$ 10.0 the signal also diminished suggesting that polymeric species may be formed, although potentiometric studies did not prove the formation of such species (Fig. 6).

The above finding, however, did not show that delocalization of the unpaired electrons of Cu^{II} over the ligand has occurred (i.e. the formation of covalent bonding).

The EPR of Cu^{II} in the presence of P or PL is almost unchanged at pH $<$ 4.4. Two ill-defined signals appeared at pH $>$ 5.0 and remained with little changes till the precipitating pH (~6.5). These results are compatible with the weak complexing properties of these ligands. On the other hand, the EPR of Cu^{II} in the presence of PM is quite different from the above described systems. The number of signals are more than four, especially at pH $>$ 4.0 with unequal intensities. The signals did not clearly display the Cu^{II} nuclear spin–electron spin coupling characteristics as in the GX–Cu^{II} system. Moreover, the signal due to the free Cu^{II} persists up to pH ~6.0. All these facts manifest the delocalization of the Cu^{II} unpaired electron over the ligand. The five signals (of symmetric unequal intensities) observed at PH $>$ 5.0 are an indication of the coupling of the nuclear spins of two nitrogens with the unpaired electron spin of the Cu^{II} (Fig. 7). One can also conclude that ligation of Cu^{II} with PM is through the nitrogen of the amino group and the oxygen of the phenoxy group as was previously predicted.[15] The coupling constant is ~61 G, which is an extra difference between PM–Cu^{II} and GX–Cu^{II} systems.

The EPR of all ternary systems are almost similar to GX–Cu^{II} binary system in having lower spin concentration in the pH range ~4.0–3.5 than other pH (Fig. 6). However, the degree of decrease in spin concentration varies with the system type, GX–P–Cu^{II} > GX–PM–Cu^{II} > GX–PL–Cu^{II}. This trend reflects the strength of ternary complex formation and/or the vitamin B_6 binary complex formation on the polymerization process of the GX–Cu^{II} binary system. On the other hand, the EPR of both GX–P–Cu^{II} and GX–PM–Cu^{II} ternary systems are almost identical in pH range ~6.0–10.0. In this range, the signals due to the nuclear spin–electron spin coupling manifests itself with almost the same spin concentration (Fig. 6). This conclusion indicates that delocalization of the unpaired electrons on the ligands did not take place. However, below pH 6 the signals are more or less similar to the PM–Cu^{II} system. There is no strong evidence that the ternary systems exhibit different EPR signals. On the other hand, the EPR of the GX–PL–Cu^{II} system is quite different in several aspects. The signals are different from that of the binary systems. There are more than four signals (5–7 signals) of quite different intensities. The upfield signals shows five splitting (three signals) which persist at pH > 3.4. The coupling constant is ~76 G. These results may be explained as due to delocalization of the unpaired electron over the ligand where possibly there is more than one nitrogen atom coordinating Cu^{II} (two or three atoms). The N nuclear spin Cu^{II} electron spin coupling leads to five or seven signals which are further split by at least two bonded hydrogen nuclei. A structure based on these speculations is shown in Fig. 1(f).

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