Biologically active pyridine mono- and bis-phosphonates: efficient ligands for co-ordination of Cu²⁺ ions

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Potentiometric and spectroscopic studies on the co-ordination ability of biologically important pyridine mono- and bis-phosphonates have shown that when sterically possible these compounds undergo tridentate co-ordination with Cu²⁺ ions. The complexes obtained are very stable. When the position of the nitrogen donor is sterically unfavourable the major binding occurs at the bis(phosphonate) site.

Our recent works on the co-ordination abilities of phosphonic analogues of carboxylic amino acids have shown that substitution of the carboxylic function by the phosphonic group changes drastically the metal binding abilities of these molecules.[1,2] Substitution of the planar, weakly basic, mono-negative carboxylate by the more bulky, tetrahedral, di-negative and more basic phosphonate has a considerable effect on the structure and stability of the metal complexes formed. It has also a critical impact on their biological activity.[3,4] Some heterocyclic dicarboxylic acids, like quinolinic acid (2,3-pyridinedicarboxylic acid) and its derivatives, are excitatory when applied to neurons.[5] These neuroactive agents resembling *N*-methyl-D-aspartate may act as agonists of neuronal receptors.[6] The replacement of the carboxylic function by the phosphonic group in a neuroactive molecule leads usually to a substance with antagonistic activity.[7]

In this work copper(II) complexes with 6-phosphonopyridine-2-carboxylic acid (H₃L¹) and -3-carboxylic acid (H₃L²), potential neuroactive compounds, and (3-methyl-2-pyridyl-amino)methylene bis(phosphonic acid) (H₄L⁴), an efficient herbicide, were studied by potentiometric and spectroscopic (UV/VIS, EPR) methods. Aminomethylene bis(phosphonic acid) (H₄L³), a detergent and anticalculus agent, was also studied for comparison with H₄L⁴.

Experimental

Phosphonic acid synthesis

The compounds H₃L¹ and H₃L² were synthesized as described earlier.[8] The bis(phosphonic acid) H₄L³ was prepared as follows: a mixture of aminodiphenylmethane (9.2 g, 0.05 mol), triethyl orthoformate (7.5 g, 0.05 mol) and diethyl phosphonate (13.8 g, 0.1 mol) was heated at 150–165 °C for 3–4 h. The volatile materials evolved were collected separately using a condenser. The remainder was treated with toluene (50 cm³) and 20% aqueous HCl (100 cm³) and then refluxed for 4 h, cooled, and the separated organic layer was removed. The remaining aqueous layer was evaporated. The residue was dissolved in hot water (100 cm³), decolorized with charcoal, filtered and evaporated again to give an oily product. The oil was dissolved in hot aqueous methanol (50 cm³) and refrigerated. The white crystals of the final product were filtered off and dried. Yield 25–30%, m.p. ≈270 °C (decomp.). NMR (D₂O): ¹H, δ 2.91 [t, *J*(CH–P) = 13 Hz]; ³¹P, δ 12.13 (s) (Found: N, 7.7; P, 31.85. Calc. for CH₇NO₆P₂: N, 7.35; P, 32.45%).

The compound H₄L⁴ was synthesized as described earlier.[9]
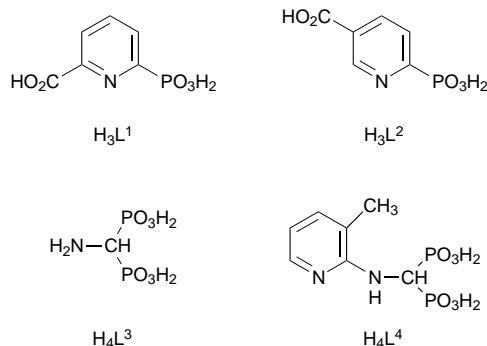
Potentiometric measurements

The purities and the exact concentrations of the stock solutions of the phosphonic acids were determined pH-metrically by the Gran method.[10] The well separated protonation steps of the phosphonate groups were used in Gran evaluation. The concentration of the copper(II) stock solution was measured gravimetrically *via* precipitation of the quinolin-8-olate. The stability constants of the proton and copper(II) complexes of the phosphonic acids were determined by pH-metric titration of 5.00 cm³ samples. The experimental parameters were as follows: phosphonic acid concentrations 0.002–0.004 mol dm⁻³; metal-ion concentrations 0.001–0.002 mol dm⁻³; metal ion to acid molar ratios 0:1, 1:1, 1:2 or 1:4; ionic strength adjusted to 0.2 mol dm⁻³ (KCl); pH range studied 2.0–11.0; duplicate pH titration calibrated in concentration;[11] pK_w = 13.76; temperature 25 ± 0.1 °C.

The pH was measured with a Radiometer pHM 64 instrument, G2040B glass and K4040 calomel electrodes, and a TTA titration unit. The concentration stability constants $\beta_{pqr} = [M_pH_qL_r]/[M]^p[H]^q[L]^r$ were calculated with the aid of the PSEQUAD computer program.[12]

Spectroscopic measurements

Visible absorption spectra were recorded with a Beckman DU 650 spectrometer, EPR spectra on a Bruker ESP 300E spectrometer at 9.2 GHz at liquid-nitrogen temperature in ethylene glycol–water (1:2 v/v) solutions, ¹H NMR spectra on a Bruker AMX 300 MHz spectrometer in D₂O solutions and UV/VIS

Table 1 Stability constants[a] of the proton (log K) and copper(II) complexes (log β) of some phosphonates at 25 °C and $I = 0.2$ mol dm^{-3} (KCl)

Parameter[b]	H₃L¹	H₃L²	H₄L³	H₄L⁴	c	d
K(HL)	7.01(1)	6.89(1)	11.03(4)	10.49(1)	10.81	10.11
K(H₂L)	3.64(2)	3.73(2)	8.85(5)	8.05(1)	6.72	5.55
K(H₃L)	≈1.0	1.93(9)	5.18(6)	5.30(1)	2.47	1.0
K(H₄L)			1.5(1)	≈1.0	≈1.0	
K(H₅L)			<1.0	<1.0		
log β[Cu(H₂L)]			23.97(2)	23.53(3)	21.80	
log β[Cu(HL)]		11.13(2)	20.18(9)	19.23(2)	17.83	
log β(CuL)	10.47(7)	7.87(2)	14.32(9)	11.59(3)	12.77	
log β(CuH₋₁L)		0.04(5)		1.66(4)	2.54	
log β[Cu(HL)₂]		20.8(2)				
log β[CuL(HL)]		18.19(4)	27.1(3)			
log β(CuL₂)	14.55(8)	14.23(3)	19.06(9)	15.12(3)	16.96	14.94
Fitting[e]	0.0037	0.0043	0.0055	0.0037		
No. of points	108	98	114	87		
pK[Cu(H₂L)]			3.79	4.30	3.97	
pK[Cu(HL)]		3.26	5.86	7.64	5.06	4.00
pK(CuL)		7.83		9.93	10.23	
log K(CuL₂)	4.08	6.36	4.74	3.53	4.19	6.65

[a] Uncertainties (three standard deviations) are given in parentheses. [b] Charges omitted for simplicity. [c] 1-Hydroxypentane-1,1-diyldiphosphonic acid.[14] [d] 1-Aminoethylphosphonic acid.[15] [e] Average difference between the calculated and the experimental titration curves expressed in cm³ of the titrant.

spectra were deconvoluted by analysis with the multipeaks Gaussian function of the Microcal ORIGIN program.[13]

Results and Discussion

6-Phosphonopyridinecarboxylic acids

Both H₃L¹ and H₃L² contain three dissociable protons within the measurable pH range (Table 1). One of the two phosphonic protons dissociates at pH < 1 and cannot be evaluated from the pH-metric data obtained here. The second acidic deprotonation site with pK of ≈1.0 for H₃L¹ and 1.93 for H₃L² seems to be localised at the carboxylic function. The pK values obtained for pyridine-2,6- or -2,5-dicarboxylic acid clearly indicate that while one of the carboxylates is strongly acidic (pK < 1 is not measurable in standard pH-metric measurements), the other is almost the same for both derivatives (pK_{CO_2H} = 2.1 and 2.17, respectively[16]). The presence of the mononegative PO₃H⁻ substituent on the pyridine ring seems to differentiate the carboxylates at positions 2 and 3, and their pK values differ by 0.8 log unit. The other two protonation constants correspond to the second phosphonate and pyridine nitrogen. An upfield shift of the signal of the proton in position 2 of the aromatic ring (H₃L²) by 0.23 ppm in the ¹H NMR spectra, following a change from pH 2 to 5, as well as the lack of such a shift between pH 5 and 7 may indicate that the pK = 3.73 corresponds to deprotonation of the pyridine ring nitrogen.

The metal complex speciations derived from potentiometric calculations for H₃L¹ and H₃L² differ considerably (Figs. 1 and 2). Only two complexes, [CuL]⁻ and [CuL₂]⁴⁻, are formed as major species in the case of H₃L¹, while for H₃L² five species are present, including three protonated complexes, [Cu(HL)], [Cu(HL)₂]²⁻ and [CuL(HL)]³⁻ (Table 1). The formation of a single equimolar [CuL]⁻ complex with H₃L¹ at pH < 2 strongly suggests tridentate co-ordination of the ligand. The sterically possible simultaneous binding of carboxylate oxygen, pyridine nitrogen and phosphonate oxygen of H₃L¹ is also reflected in a very high stability constant (log K = 10.47) of the [CuL]⁻ complex. Owing to this fact the proton-displacement reaction Cu²⁺ + H₂L⁻ ⇌ [CuL]⁻ + 2H⁺ is shifted strongly to the right at the starting pH value even in an equimolar solution [Cu²⁺ is ≈10% of the total copper(II) concentration]. This is reflected in the rather high uncertainties in the log β values (see Table 1), although the speciation model is quite simple. In the case of H₃L² the carboxylate group is separated from the chelating
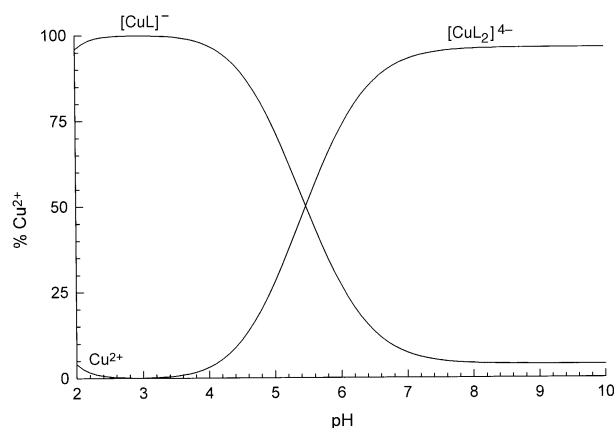

Fig. 1 Species distribution curves for the complexes formed in the copper(II)–H₃L¹ system as a function of pH; $c_{Cu} = 0.002$ mol dm^{-3}, $c_{H_3L^1} = 0.006$ mol dm^{-3}
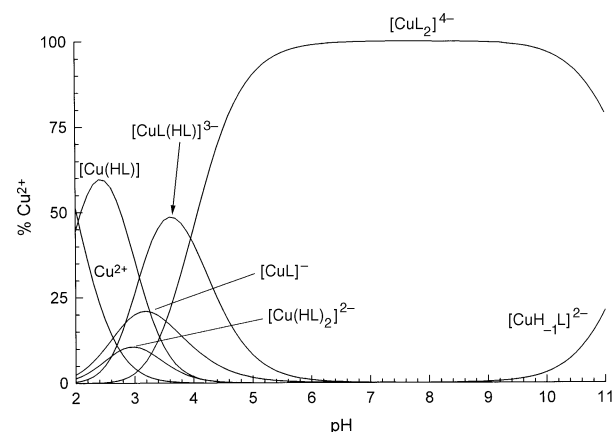

Fig. 2 Species distribution curves for the complexes formed in the copper(II)–H₃L² system as a function of pH; $c_{Cu} = 0.002$ mol dm^{-3}, $c_{H_3L^2} = 0.006$ mol dm^{-3}

function and is unable to co-ordinate the metal ion simultaneously, due to steric reasons. Thus, in the complex [Cu(HL)] the Cu²⁺ ion binds to the {N_py, PO₃²⁻} chelate donor system and the protonated carboxylate is unbound. Deprotonation of this species leads to the formation of [CuL]⁻. The stability constant of [CuL]⁻ species with H₃L¹ is 2.6 log units lower than that

Table 2 Spectroscopic parameters for copper(II) complexes formed by some phosphonates

Ligand	Species	EPR $A_\parallel$/G	g	UV/VIS λ/nm (ε/dm^3 mol^{-1} cm^{-1})
H$_3$L^1	[CuL]$^-$	101	$g_\parallel$ = 2.311	772(46) [a]
	[CuL$_2$]$^{4-}$	104	$g_\parallel$ = 2.315	781(48) [a]
H$_3$L^2	[Cu(HL)]	149	$g_\parallel$ = 2.305	705(69) [a]
	[CuL(HL)]$^{3-}$	157	$g_\parallel$ = 2.290	
	[CuL$_2$]$^{4-}$	166	$g_\parallel$ = 2.281	679(53) [a]
H$_4$L^3	[Cu(H$_2$L)] [b,c]			287(1196) [d]
				743(84) [a]
	[Cu(HL)]$^-$ [b,c]			289(1883) [d]
				735(63) [a]
	[CuL]$^{2-}$	—	$g_\parallel$ = 2.041	686(45) [a]
			$g_\perp$ = 2.154	
	[CuL$_2$]$^{6-}$	171	$g_\parallel$ = 2.255	657(57) [a]
H$_4$L^4	[Cu(H$_2$L)] [b]			309(6226) [e]
				800(23) [a]
	[Cu(HL)]$^-$ [b]			310(5780) [e]
				750(41) [a]
	[CuL]$^{2-}$ [b]			310(5318) [e]
				737(32) [a]
	[CuH$_{-1}$L]$^{3-}$	128	$g_\parallel$ = 2.389	309(4055) [e]
				722(32) [a]
	[CuL$_2$]$^{6-}$ [b]			309(4689) [e]
				734(33) [a]

[a] d–d Transition. [b] Unresolved and very broad EPR spectrum. [c] Some precipitation was observed. [d] NH$_2$→Cu^{2+} charge-transfer transition. [e] Intrapyridine transition.

of H$_3$L^2 (Table 1), confirming the tridentate co-ordination of the latter and the bidentate binding of the former. It is interesting that the log K value for the reaction [Cu(HL)] $\rightleftharpoons$ [CuL]$^-$ + H$^+$ is much higher (3.26) than the carboxylate pK of the free H$_3$L^2 (1.93). The metal ion co-ordination to the {N$_{py}$, PO$_3^{2-}$} donor set leads to complete deprotonation of both groups involved in metal binding and a strong increase in the negative charge on the pyridine ring. This may be the main cause for a distinct increase in the protonation constant of the 3-CO$_2^-$ group in the [Cu(HL)] species. At first glance, the similar stability constants obtained for the bis complexes with both H$_3$L^1 and H$_3$L^2, log β = 14.55 and 14.23, respectively, could suggest a similar binding mode in these [CuL$_2$]$^{4-}$ species.

The spectroscopic data (both absorption and EPR) are, however, distinctly different, indicating different geometries and different donor sets involved in metal ion co-ordination. The tridentate co-ordination of the {CO$_2^-$, N$_{py}$, PO$_3^{2-}$} donor set is possible only when one donor is bound to the metal ion axially. This may lead to a strongly distorted square-pyramidal rather than a tetragonal geometry with unusual EPR parameters (very low $A_\parallel$ and relatively high $g_\parallel$,[2] Table 2). Low $A_\parallel$ values are observed for both H$_3$L^1 species, [CuL]$^-$ and [CuL$_2$]$^{4-}$, indicating that also in the bis complex tridentate co-ordination of at least one ligand occurs (Table 2). The $A_\parallel$ and $g_\parallel$ values for the [CuL]$^-$ and [CuL$_2$]$^{4-}$ complexes of H$_3$L^2 are similar to those for simple tetragonal systems with one or two nitrogens involved in the metal-ion binding.[1,2,17] The variations of the d–d transition energies also support this. The simple chelation of H$_3$L^2 by {N$_{py}$, PO$_3^{2-}$} shifts the d–d transition band towards higher energies expected for 1N ([CuL]$^-$) or 2N ([CuL$_2$]$^{4-}$) co-ordination,[1,2,17] while tridentate co-ordination changes d–d transitions only slightly when compared to those of Cu^{2+}(aq) ($\approx$800 nm). The apical co-ordination diminishes the high-energy shift caused by the equatorial nitrogen binding in both the mono and bis complexes with H$_3$L^1. Thus, the similarity in stability constants of the bis complexes is rather accidental. The low value of log K_2 for H$_3$L^1 (log K_2 = 4.08) suggests a bidentate axial–equatorial co-ordination of the second ligand. For H$_3$L^2 this log K_2 value is much higher (6.36), as the bidentate chelation of the first ligand in [CuL]$^-$ leaves enough co-
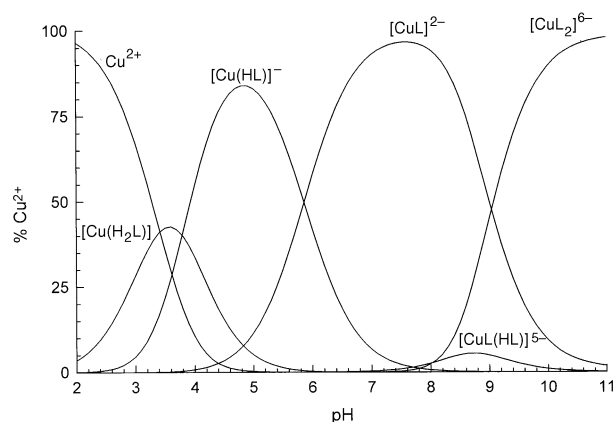

Fig. 3 Species distribution curves for the complexes formed in the copper(II)–H$_4$L^3 system as a function of pH; c_{Cu} = 0.002 mol dm^{-3}, $c_{H_4L^3}$ = 0.006 mol dm^{-3}

ordination sites free in the equatorial plane for a much stronger equatorial chelation of the second ligand.

Bis(phosphonic acids)

The compound H$_4$L^3 has five protonation sites (Table 1) with a relatively basic amino nitrogen (pK = 11.03). The high basicity of this donor stems from the high negative charge of the two deprotonated dinegative phosphonic groups. The protonation constants for both phosphonic functions correspond well to those found for other bis(phosphonic acids) by taking into account the electron-attracting effect of the nearby NH$_3^+$ group, which slightly increases the acidity of these groups.[14]

During the titration of the Cu^{2+}–H$_4$L^3 system at a 1:1 metal ion to phosphonic acid ratio slight precipitation was observed in the acidic pH range. The precipitate is most likely a neutral phosphonato complex. In order to obtain reliable pH-metric data over as wide a pH range as possible, back titrations with 0.2 mol dm^{-3} HCl starting from pH 11 were also carried out. In this way the precipitation down to pH 3 could be avoided even under equimolar conditions, probably due to the formation of oversaturated solutions. The joint evaluation of the pH titration data obtained in this way indicates the formation of four major species, [Cu(H$_2$L)], [Cu(HL)]$^-$, [CuL]$^{2-}$ and [CuL$_2$]$^{6-}$ and one minor complex [CuL(HL)]$^{5-}$ (Fig. 3). The complex [CuL]$^{2-}$ dominating at pH $\approx$ 8 is very likely to contain a tridentate co-ordinated ligand through the amino and the two phosphonate donors. Both the d–d transition (686 nm) and the EPR parameters (Table 2) suggest tetragonal geometry around Cu^{II} and 1N binding in the equatorial plane. The tridentate co-ordination of the ligand is clearly reflected in the much higher stability of the complex when compared to that of the bidentate {NH$_2$, PO$_3^{2-}$}-co-ordinated [CuL] complex of the phosphonic derivative of α-alanine (see Table 1). The complex can bind two protons with log K([CuL]$^{2-}$) = 5.86 and log K\{[Cu(HL)]$^-$\} = 3.79. These stepwise protonation processes are accompanied by a significant shift of the d–d transition to $\approx$740 nm, which indicates a continuous rearrangement of the tridentate {NH$_2$, PO$_3^{2-}$, PO$_3^{2-}$} bonding mode to a mixture of linkage isomers having bidentate co-ordination. Accordingly, the equilibrium between the two isomers of [Cu(H$_2$L)], namely {NH$_2$, PO$_3$H$^-$} with the other phosphonate group monoprotonated and {PO$_3^{2-}$, PO$_3$H$^-$} with the amino group protonated, is probably shifted towards the former species. This may be supported by the d–d transition at around 743 nm, while for a species with two monoprotonated phosphonic functions this should be shifted to around 800 nm.[14] The formation of the [CuL$_2$]$^{6-}$ complex at pH > 8 leads to a distinct shift in the d–d transition to higher energies (from 686 to 657 nm). This indicates that two nitrogens are involved in co-ordination. The parameters of the EPR spectra suggest a tetragonal geometry around Cu^{2+} in the bis
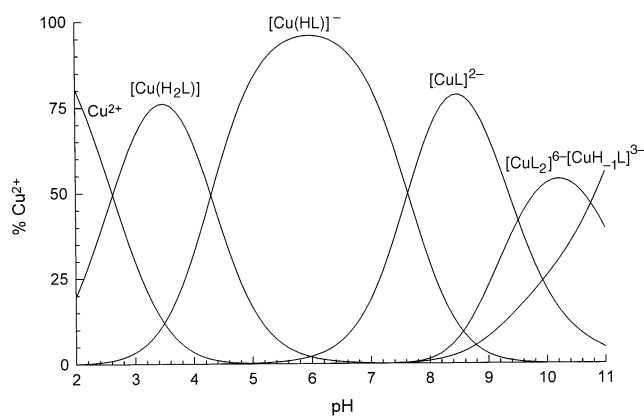
Fig. 4 Species distribution cuves for the complexes formed in the copper(II)–H_4L^4 system as a function of pH; $c_{Cu} = 0.002$ mol dm^{-3}, $c_{H_4L^4} = 0.006$ mol dm^{-3}

complex, and thus the binding mode is based on the formation of two effective {NH_2, PO_3^{2-}} chelate rings. Some apical interaction of the second phosphonate cannot be excluded.

The compound H_4L^4 also has five protonation sites, four of them at the two phosphonic groups and one at the pyridine nitrogen. The lack of an adjacent positively charged amino group results in high values of the first and second phosphonic protonation constants {log $K([HL]^{3-}) = 10.49$ and log $K([H_2L]^{2-}) = 8.05$}, while the other two {log $K(H_4L)$ and log $K([H_5L]^+)$} are similar to those observed for H_4L^3 (Table 1). High protonation constants for the phosphonic groups are usually observed for bis(phosphonates).[14]

The set of complex species calculated from potentiometric titrations for Cu^{2+}–H_4L^4 up to pH 8 is similar to that discussed above for Cu^{2+}–H_4L^3 (Figs. 3 and 4). The stability constants of the [Cu(H$_2$L)], [Cu(HL)]$^-$ and [CuL]$^{2-}$ complexes formed, are however distinctly lower than those for the corresponding H_4L^3 complexes (Table 1). The log β values of the [CuL]$^{2-}$ species of H_4L^3 (14.32) and that of H_4L^4 (11.59) indicate that the pyridine-N donor of H_4L^4, if bound at all, is much less effective in metal binding than is the amino group of H_4L^3. This is due partly to its lower basicity (Table 1) and its unfavourable position within the molecule. The lesser binding ability of H_4L^4 compared to H_4L^3 could also be attributed to steric hindrance by the phenyl moiety to amine N co-ordination. Accordingly, in the case of the H_4L^4 complexes the proposed equilibrium between isomers (see above) is shifted strongly to the purely phosphonate-chelated species, *e.g.* the d–d transition at 800 nm with low ε value observed for [Cu(H$_2$L)] strongly suggests the binding of two protonated phosphonic functions (see above and ref. 14). Both the stability data and absorption spectra support this assumption also for other species (see Table 2). The d–d transition energy of 740–750 nm for the [Cu(HL)]$^-$ and [CuL]$^{2-}$ complexes and very low ε values when compared to nitrogen-bound complexes (see *e.g.* complexes with H_4L^3 in Table 2) correspond to chelation of one or two deprotonated phosphonic groups.[14,18] Further, the basicity-adjusted equi-

librium constants (log K^*), which take into account the differences in the basicity of the co-ordinating donors, and characteristic of the reaction Cu^{2+} + [H$_3$L]$^-$ $\rightleftharpoons$ [CuL]$^{2-}$ + 3H$^+$ for H_4L^4 and 1-hydroxypentane-1,1-diyldiphosphonic acid [only able to participate in bis(phosphonate) chelation], agree fairly well with each other (log $K^* = -8.05$ for H_4L^4 and -8.23 for the pentane derivative). The less effective binding properties of the pyridine N are reflected in the less favoured formation of the [CuL$_2$]$^{6-}$ complex. As seen in Fig. 4, besides the bis complex a deprotonated 1:1 complex [CuH$_{-1}$L]$^{3-}$ is also formed in significant concentration in the Cu^{2+}–H_4L^4 system. The deprotonation leading to this species at pH $\approx$ 9 corresponds to the ionisation of a metal-bound water molecule; log K of 9.93 is expected for this type of hydrolysis reaction.[19] Accordingly, the much weaker co-ordination of the second H_4L^4 molecule (log $K([CuL_2]^{6-}) = 3.53$ *vs.* 4.74 for H_4L^3) will not prevent hydrolytic reactions, namely the formation of mixed-ligand hydroxo complexes.

References

1 T. Kiss, M. Jezowska-Bojczuk, H. Kozłowski, P. Kafarski and K. Antczak, *J. Chem. Soc., Dalton Trans.*, 1991, 2275.
2 M. Jezowska-Bojczuk, T. Kiss, H. Kozłowski, P. Decock and J. Barycki, *J. Chem. Soc., Dalton Trans.*, 1994, 811.
3 P. Kafarski and P. Mastalerz, *Beitr. Wirkst. Forsch.*, 1984, **21**, 1.
4 W. Bal, I. Bertini, H. Kozłowski, R. Monnani, A. Scozzafava and Z. Siatecki, *J. Inorg. Biochem.*, 1990, **40**, 227.
5 T. W. Stone and M. N. Perkins, *Eur. J. Pharm.*, 1985, **72**, 411.
6 M. N. Perkins and T. W. Stone, *Brain Res.*, 1983, **259**, 259.
7 T. W. Stone, *Br. J. Pharmacol.*, 1984, **81**, 175.
8 B. Boduszek, *J. Prakt. Chem.*, 1992, **334**, 444.
9 F. Suzuki, T. Ohya, T. Ikai and T. Oguchi, *Ger. Pat.*, 831 578, 1979.
10 G. Gran, *Acta Chem. Scand.*, 1950, **4**, 559.
11 H. Irving, M. G. Miles and L. D. Pettit, *Anal. Chim. Acta*, 1967, **38**, 475.
12 L. Zékány and I. Nagypál, in *Computational Methods for the Determination of Stability Constants*, ed. D. Leggett, Plenum, New York, 1985.
13 Microcal ORIGIN, version 3.5, Microcal Software Inc., Northampton, MA, 1991–1994.
14 M. Dyba, M. Jezowska-Bojczuk, E. Kiss, T. Kiss, H. Kozłowski, Y. Leroux and D. El Manouni, *J. Chem. Soc., Dalton Trans.*, 1996, 1119.
15 T. Kiss, J. Balla, G. Nagy, H. Kozłowski and J. Kowalik, *Inorg. Chim. Acta*, 1987, **138**, 25.
16 R. M. Smith and A. E. Martell, *Critical Stability Constants*, Plenum, New York, 1975.
17 L. D. Pettit, J. Gregor and H. Kozłowski, in *Perspectives on Bioinorganic Chemistry*, JAI Press, London, 1990.
18 H. Kozłowski and G. Micera, in *Handbook of Metal–Ligand Interactions in Biofluids, Bioinorganic Chemistry*, ed. G. Berthon, Marcel Dekker, New York, 1995, p. 566.
19 H. Siegel and R. B. Martin, *Chem. Rev.*, 1982, **82**, 385.