Copper Anti-inflammatory Drugs in Rheumatoid Arthritis. Part 3.[1] A Potentiometric and Spectroscopic Study of Zinc(II), Calcium(II), and Magnesium(II) Polyaminodicarboxylate Complexes

Graham E. Jackson * and Mark J. Kelly
Department of Chemistry, University of Cape Town, Rondebosch 7700, South Africa

Zinc(II), calcium(II), and magnesium(II) complexes of 3,6,9,12-tetra-azatetradecanedioic acid and 3,6,9-triazaundecanedioic acid have been studied at 25 °C and an ionic strength of 0.15 mol dm^{-3} NaCl, using glass-electrode potentiometry. N.m.r. spectroscopy has been used to rationalize the data in terms of solution structures. Computer simulation has been used to predict the effect of the ligands on the blood plasma metal-ion distribution *in vivo*.

As part of an on-going study into the design of copper(II)-based antirheumatic drugs[1,2] we have recently synthesized 3,6,9,12-tetra-azatetradecanedioic acid [H_2ttda, $HO_2C(CH_2NHCH_2)_4CO_2H$] and 3,6,9-triazaundecanedioic acid [H_2dtda, $HO_2C(CH_2NHCH_2)_3CO_2H$].[3] Preliminary studies on the copper(II) complexing ability of these two ligands has indicated their potential usefulness as antirheumatic drugs.[2] Computer simulation, however, has shown that the high *in vivo* concentration of zinc(II) and calcium(II) make these two metal ions important. It is for this reason that we have studied the solution thermodynamics of ttda and dtda with these two metal ions. For comparison magnesium(II) has been included in this study.

Theory

Throughout the analysis of the data we have made extensive use of certain features within the ESTA suite of programs.[4] Some of these features are fairly novel and require some explanation. The deprotonation function $\bar{Q}$ is the average number of protons released per metal ion as a result of complexation and is defined according to equation (1), where T_H and T_M are the total proton

$$\bar{Q} = (T_H^* - T_H)/T_M \quad (1)$$

and metal concentrations respectively; T_H^*, given by equation (2), is the calculated total concentration of protons that would

$$T_H^* = [H] - [OH] + \Sigma r\beta_{0qr}[L]^q[H]^r \quad (2)$$

be necessary to give rise to the observed pH if no complexation took place. The summation is over all protonated ligand species. In order to evaluate T_H^* it is necessary to solve for the free-ligand concentration using equation (3). If we define a formation function for the ligand subsystem according to equation (4) then F, the average number of dissociable protons in a complex (assuming that it is the predominant complex) is given by equation (5). Here $\bar{Q}$ represents the average number of protons

$$T_L = [L] + \Sigma q\beta_{0qr}[L]^q[H]^r \quad (3)$$

$$\bar{n} = (T_H^* - [H] + [OH])/T_L' \quad (4)$$

$$F = q\bar{n} - \bar{Q}p \quad (5)$$

released from the ligand as a result of complexation, while $\bar{n}$ is the average number of protons which would be bound to the ligand in the absence of metal complexation. The difference between the two therefore gives the average number of dissociable protons remaining on the ligand after complexation. Clearly, cognizance of metal–ligand stoicheiometries has to be taken.

The formation function $\bar{Z}$, which is the average number of ligands bound per metal ion, is given by equation (6). The

$$\bar{Z} = (T_L - [L])/T_M \quad (6)$$

$$\text{p.m.i.} = \frac{\text{concentration of l.m.w. species in presence of drug}}{\text{concentration of l.m.w. species in absence of drug}} \quad (7)$$

plasma mobilizing index (p.m.i.),[5] which is a measure of the effect a drug has on the concentration of low-molecular-weight (l.m.w.) species is defined according to equation (7).

Results and Discussion

The protonation constants and protonation schemes for these two ligands have been presented before.[2] The formation curves obtained for the Zn–ttda and Zn–dtda systems at 25 °C and I = 150 mmol dm^{-3} NaCl overlap and indicate simple, stepwise, mononuclear complexation under the conditions of this study. The deprotonation function, $\bar{Q}$, of the Zn–ttda system, however [Figure 1(*a*)], is not as simple, indicating the presence of protonated species. This illustrates the complementary nature of these two functions. The formation function is sensitive to the number of co-ordinated metal ions, while the deprotonation function is sensitive to the number of protons (or hydroxyl ions) in the complex. Analysis of the data confirms the above observations. The Table lists the results for the major metal–ligand (ML) and the minor protonated M(HL) species.

Chang and Douglas[6] have studied an analogous ligand, triethylenetetramine-*N'*,*N''*-diacetic acid (H_2trienda), in which the acetate substituents are on the central, rather than terminal nitrogens, as in our case. The constants reported for the zinc(II) complexes of this analogue are substantially different from ours (see Table). It is difficult to rationalize these discrepancies in terms of the structural differences between the two ligands, especially as the proton and copper(II) complex-formation constants of ttda and trienda are in much closer agreement.[2]

Inspection of thermodynamic data[7] for zinc(II) complexes of related ligands shows that enthalpy factors make the biggest contribution towards the stability of amino complexes. The neutralization of charge and hence decrease in hydration is most likely responsible for the importance of entropy in determining

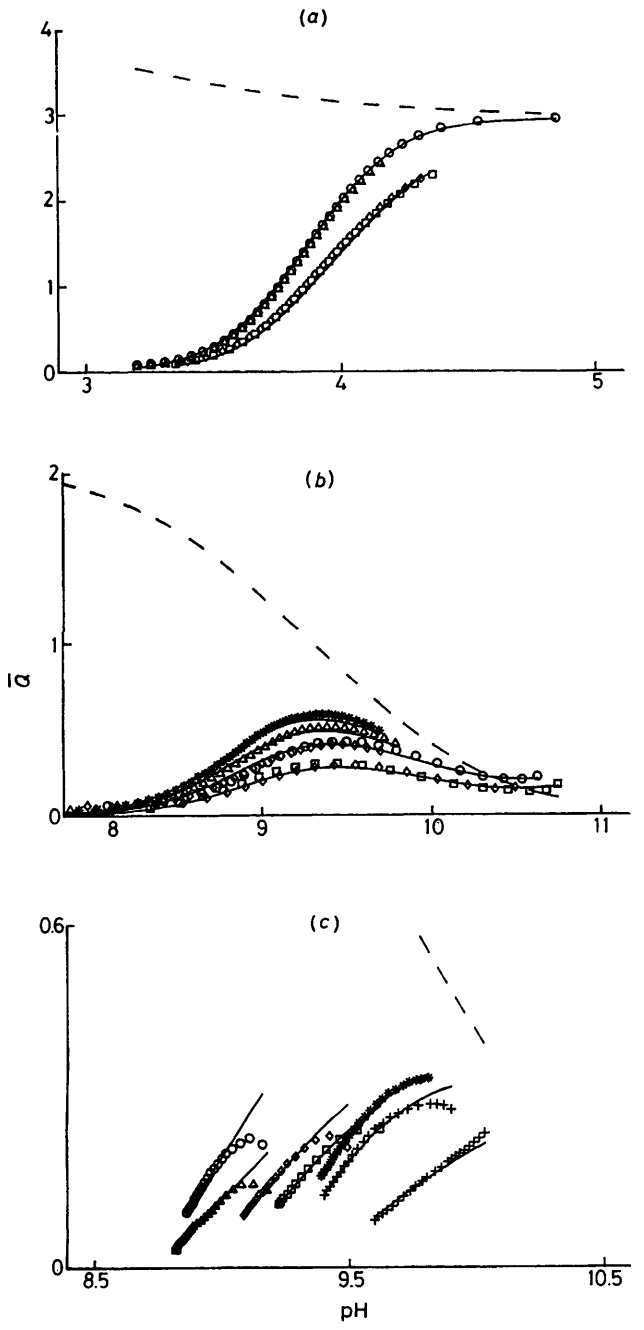

Figure 1. Experimental deprotonation curves for the systems (*a*) Zn–ttda {[ttda], [Zn] = 3.94, 1.96 (○) and 1.95, 1.96 (□, ◇) mmol dm^{-3}}, (*b*) Ca–ttda {[ttda], [ca] = 1.75, 1.77 (◇, □); 3.1, 1.6 (○); 2.8, 1.4 (△); and 5.0, 1.4 (✱) mmol dm^{-3}}, and (*c*) Mg–ttda {[Mg] = 0–32, [ttda] = 6.3 (+); 12.0 (✱); 8.3 (□); 7.8 (◇); 7.2 (△); and 13.4 (○) mmol dm^{-3}} at 25 °C and I = 150 mmol dm^{-3} NaCl. The solid lines represent the theoretical curves calculated using the formation constants given in the Table. The broken lines are the $\bar{n}$ curves

the stability of acetate ligand complexes. The presence of two acetate groups on ethylenediamine (en) lowers ΔH° for zinc(II) complexation by *ca.* 4 kJ mol^{-1} [ΔH°(en) = −29.3, ΔH°-(ethylenediamine-*N*,*N*′-diacetate) = −25.5 kJ mol^{-1}], hence we estimate ΔH° for zinc complexation of ttda to be −33.2 kJ mol^{-1} [ΔH°(triethylenetetramine) = 37.2 kJ mol^{-1}]. Similarly ΔH° for zinc complexation of dtda is estimated to be −23.2 kJ mol^{-1} [ΔH°(diethylenetriamine) = −27.2 kJ mol^{-1}]. At the same time the data show that ΔS° increases by *ca.* 30 J K^{-1} mol^{-1} per acetate group. Hence ΔS° is estimated to be *ca.* 165 J K^{-1} mol^{-1} for zinc(II) complexation of ttda and dtda. This leads to values for ΔG° of −82.4 and −72.4 kJ mol^{-1} and equilibrium constants of $10^{14.4}$ and $10^{12.7}$ at 25 °C for the formation of [Zn(ttda)] and [Zn(dtda)] respectively. This is in reasonable agreement with the values of 15.65 and 13.19 obtained for log β_{110} in this study.

The formation curves for the Ca–ttda system are superimposable, indicating that the major species formed are simple mononuclear complexes. At high pH the formation curves fan back, which is characteristic of hydroxy species formation. This is shown more clearly by the deprotonation function [Figure 1(*b*)]. Computer analysis of the data confirms the presence of only two species, ML and MLH_{-1}. The 'goodness of fit' between the experimental data and the proposed model, given in the Table, is indicated by the solid lines in Figure 1(*b*). Similar results were obtained for the Ca–dtda, Mg–ttda, and Mg–dtda systems. The deprotonation curves for the Mg–ttda system are shown in Figure 1(*c*).

A comparison of the results obtained for the calcium(II) and magnesium(II) systems is interesting. The [Mg(dtda)] complex is more stable than the [Mg(ttda)] complex. Presumably this is due to the small size of the magnesium(II) ion. For the larger calcium(II) ion the expected order of complex stability [Ca(ttda)] > [Ca(dtda)] is seen.

Ionic radius is a common theme running through the co-ordination chemistry of calcium(II) and magnesium(II).[8] The higher charge density of magnesium(II) tends to make complexes of this metal ion more stable than the corresponding calcium(II) complexes. On the other hand the small size of the magnesium ion makes multidentate co-ordination difficult. The results of this study are consistent with these observations. The [Ca(dtda)] complex has the same stability as [Mg(dtda)], while [Ca(ttda)] is more stable than [Mg(ttda)].

The potentiometric results are supported by an n.m.r. study of the Ca–dtda, Ca–ttda, and Zn–ttda systems. Protonation results for the free ligands have been reported previously.[2] Figure 2 shows the ^{13}C n.m.r. chemical shifts of the Ca–dtda system as a function of pD. Over the entire pD range only single resonances are observed for non-equivalent carbon atoms, indicating that the ligand is in rapid exchange amongst the various possible isomers. Both the ^{1}H and ^{13}C n.m.r. titration curves level off at pD 10 indicating that formation of the [Ca(dtda)] complex is complete. This is reflected in the associated species distribution diagram. However, beyond pD 10 substantial formation of the $[Ca(dtda)(OH)]^{-}$ complex occurs. This is not reflected in the n.m.r. titration curves, presumably because the ligand chemical shifts are insensitive to hydrolysis of the metal.

Figure 3 shows the variation in ^{13}C n.m.r. chemical shift of dtda and ttda as a function of calcium concentration. These curves indicate that complexation is complete after the addition of 1 equivalent of calcium(II). The addition of excess of calcium(II) does not result in further complexation. It is possible to analyse, using the method of Hague and Moreton,[9] the titration curves for the stability of the complex formed, values of $10^{2.2}$ and $10^{3.2}$ being obtained for β_{110} of dtda and ttda respectively. Given the variation in ionic strength these results are in excellent agreement with the potentiometric results for β_{110}. It is interesting that the chemical shifts of C^5 (dtda) and C^7 (ttda) are insensitive to complexation, suggesting that the central nitrogens are not involved in complexation.

Assignment of the ^{13}C n.m.r. spectra of ttda followed from the assignment of the ^{1}H n.m.r. spectra[2] *via* a two-dimensional heteronuclear correlation (HETCOR) experiment[10] (Figure 4). This showed that the ^{13}C chemical shifts were not in the same order as the ^{1}H chemical shifts, C^7 being shifted to lower field. A careful examination of the calcium(II) n.m.r. titration curves revealed a crossover of the C^4 and C^5 resonances of dtda and the

Table. Formation constants determined in this study at 25 °C, I = 150 mmol dm^{-3} NaCl; σ donates standard deviation in log β and n is the number of experimental observations used as data in the least-squares calculations; R is the Hamilton R factor. The general formula of a complex is expressed by $M_pL_qH_r$

M	L	p	q	r	log β_{pqr}	σ	n	R
Zn^{II}	dtda	1	1	0	13.19	0.004	324	0.003
		1	1	1	16.72	0.02		
	ttda	1	1	0	15.65	0.004	112	0.004
		1	1	1	19.14	0.02		
Ca^{II}	dtda	1	1	0	2.64	0.02	240	0.005
		1	1	−1	−8.53	0.04		
	ttda	1	1	0	3.17	0.007	216	0.008
		1	1	−1	−8.19	0.04		
Mg^{II}	dtda	1	1	0	2.62	0.003	694	0.004
	ttda	1	1	0	2.34	0.003	779	0.004
		1	1	−1	−8.54	0.04		
Zn^{II}	trienda[4]	1	1	0	19.13			
		1	1	1	24.66			
		1	1	2	28.01			

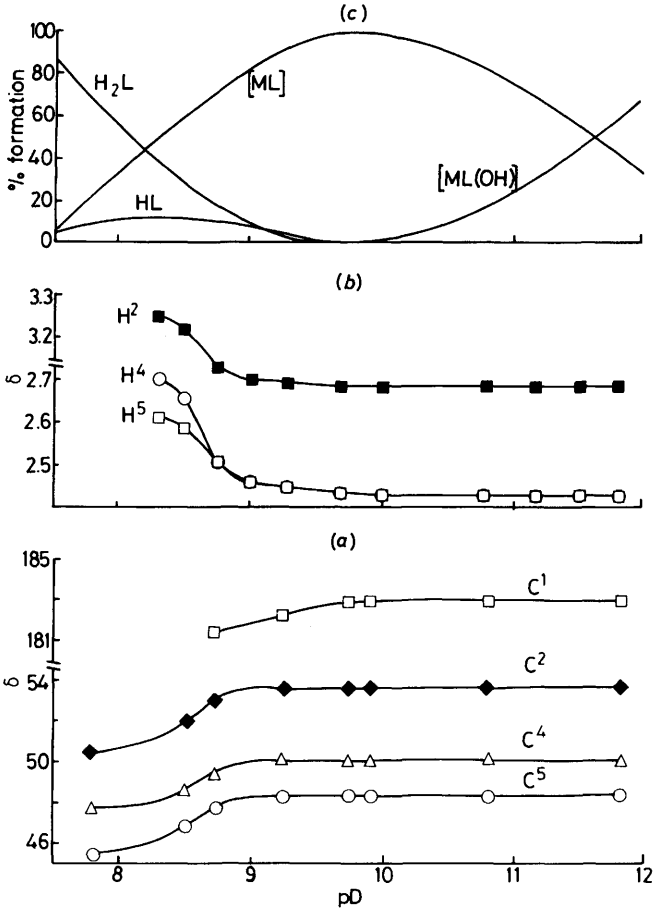

Figure 2. pD Dependence of the ^{1}H (*a*) and ^{13}C (*b*) n.m.r. chemical shifts of dtda in the presence of calcium(II). [dtda] = 0.24, [Ca^{II}] = 0.48 mol dm^{-3}. (*c*) Species distribution calculated for the above system using the constants given in the Table

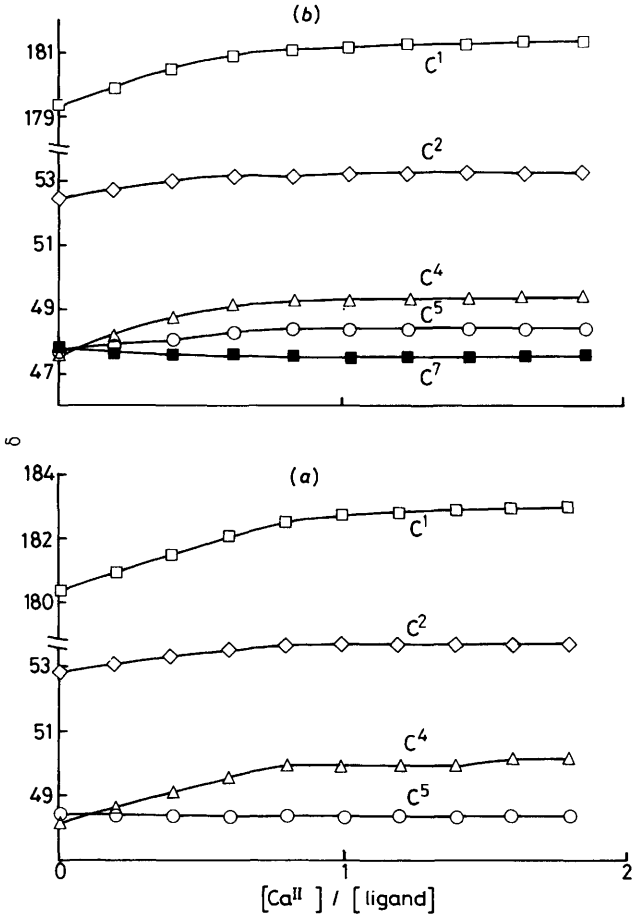

Figure 3. Carbon-13 n.m.r. chemical shifts as a function of calcium(II) concentration for (*a*) dtda and (*b*) ttda at pD 10.0

C^7, C^4, and C^5 resonances of ttda. A similar crossover is seen in the chemical shifts of 3,7-diazanonane-1,9-diamine.[11]

Attempts to study the solution structure of [Zn(ttda)] using ^{1}H and ^{13}C n.m.r. spectroscopy were complicated by the formation of several isomers in solution, and by the line broadening associated with exchange between these isomers. However, while a complete assignment of the spectra (Figure 5) could not be made it was possible to draw a number of conclusions about the structure of the [Zn(ttda)] and $[Zn(Httda)]^+$ complexes in solution. At low pD, signals attributable to the free ligand are seen as indicated [Figure 5(*a*)]. Two very broad signals are seen for H^2 and H^4, H^5, H^7. This spectrum is typical of protonated polyaminopolycarboxylate complexes, with protonation at the terminal nitrogen.[12] Since separate signals are seen for both free and complexed ttda, exchange between these two forms of the ligand must be slow on the n.m.r. time-scale.

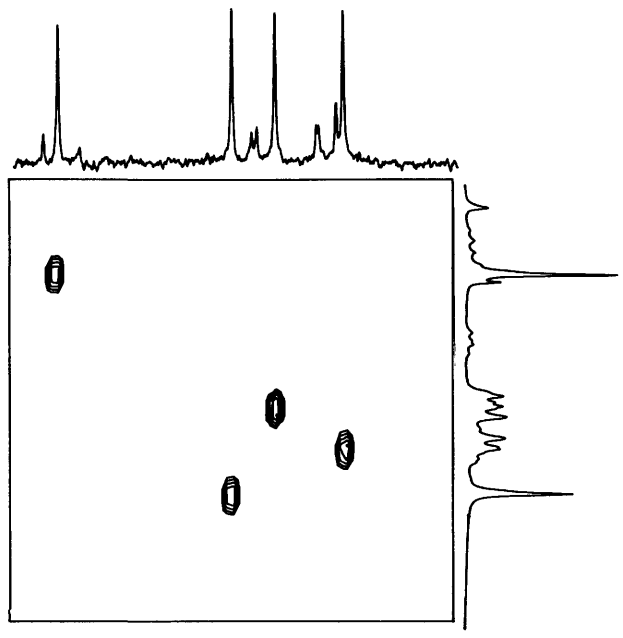

Figure 4. N.m.r. spectrum of ttda at pD 6.0 obtained using the HETCOR pulse sequence with J_{CH} = 140 Hz

As the pD is raised the ^{1}H n.m.r. signals move to higher field and gain fine structure. The major spectral changes occur between pD 3 and 5 which corresponds to the measured pK_a for $[Zn(Httda)]^+$ of 3.5. At pD 8 the [Zn(ttda)] complex is fully formed. At this pD the signals attributable to the acetate groups (H^2) consist of several broad peaks and a number of AB quartets (J_{AB} = 7 Hz). The non-equivalence of these geminal protons and hence the AB pattern is a clear indication that the acetate group is co-ordinated to the metal and that exchange of these acetate groups amongst the various possible isomers is slow on the n.m.r. time-scale. The ABCD nature of H^4 and H^5, together with the slow interconversion between different geometric isomers, results in the broad signals between δ 2.4 and 3.2. The existence of several geometric isomers in solution is confirmed by the ^{13}C n.m.r. spectrum [Figure 5(*b*)]. Here again signals attributable to the free ligand are seen.

An important factor in determining the copper(II) p.m.i.[5] of a ligand is its affinity for zinc(II) and calcium(II). These two metal ions are present, *in vivo*, at a much higher concentration than copper(II), and hence their complexes often predominate in solution. This is the reason why ethylenediamine-*N*,*N*,*N*′,*N*′-tetra-acetate does not mobilize copper(II) *in vivo*.[1] The zinc(II) and copper(II) p.m.i. curves for ttda and dtda are shown in Figure 6. At a total ligand concentration of 10^{-5} mol dm^{-3} the calcium(II) p.m.i. is <0.01. These curves indicate that ttda and dtda should have very little effect on the *in vivo* calcium(II) distribution but should cause some redistribution of zinc(II). The ligands should therefore be efficient mobilizers of copper(II).

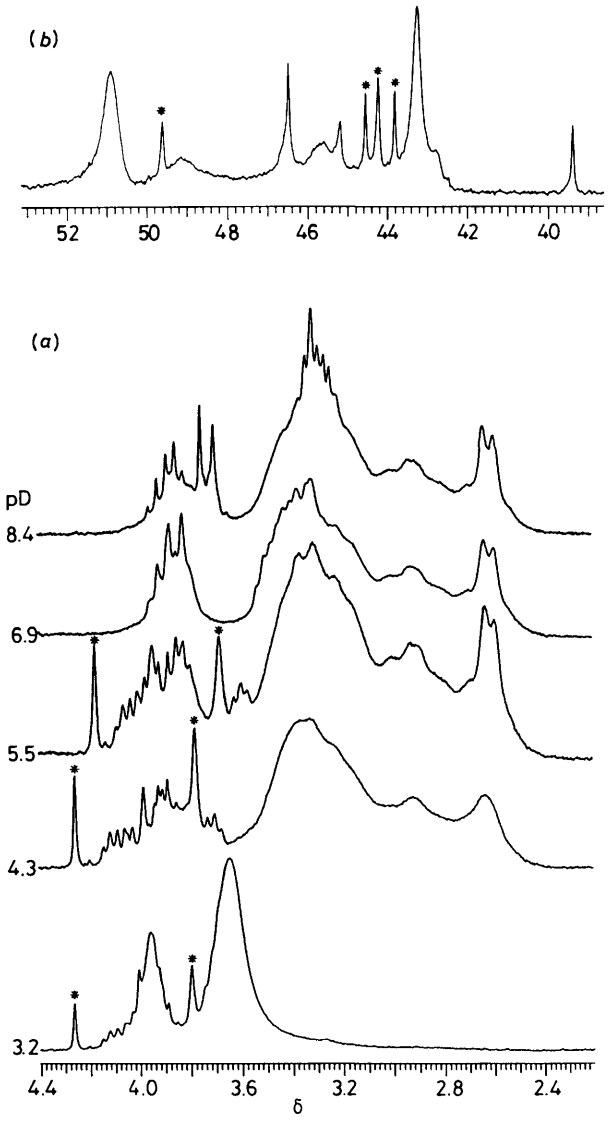

Figure 5. Proton (*a*) and ^{13}C (*b*) n.m.r. spectra of Zn–ttda as a function of pD. [ttda] = [Zn^{II}] = 0.1 mol dm^{-3}. Starred peaks are assigned to the free ligand

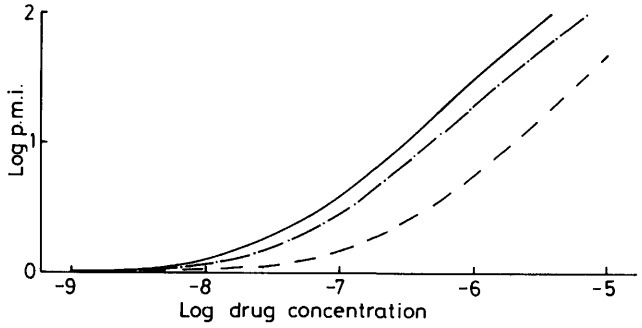

Figure 6. Zinc(II) (– – – –) and copper(II) p.m.i. curves for ttda (———) and dtda (– · – · –)

Experimental

The compounds H_2ttda and H_2dtda were prepared as the hydrochloride salts as described previously[3] using standard methods. Because of the tendency of these ligands to form lactams in acid solution, the titration procedure described previously was used.[2]

Potentiometric titrations were carried out in a double-walled vessel, thermostatted at 25 °C. Measurements were made on a Radiometer PHM84 research pH meter equipped with a Metrohm glass electrode and a calomel reference electrode with a renewable liquid junction of 150 mmol dm^{-3} NaCl. Electrode calibration was according to the method of May *et al.*[13]

Data were analysed on a Sperry Univac 1100 computer using the ESTA suite of programs.[4] Refinement of parameters was based on unweighted e.m.f. measurements and the Hamilton *R* factor defined according to equation (8), where E_i^o and E_i^c are

$$R = [\Sigma(E_i^o - E_i^c)^2]^{\frac{1}{2}}[(N - n_p)\Sigma(E_i^o)^2]^{-\frac{1}{2}} \quad (8)$$

the measured and calculated e.m.f. values for each of N titration points and n_p is the number of parameters refined.

The program MAGEC[13] was used to determine pK_w from strong acid–strong base titrations. A value of -13.76 was obtained and used throughout. N.m.r. spectra were recorded in D_2O solution on a Varian VXR200 spectrometer using t-butyl alcohol as an internal reference. The pD = pH + 0.4 of the solutions was measured using a microcombination electrode and adjusted using NaOD or DCl.

Acknowledgements

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