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Kinetic studies on the reactions of macrocyclic complexes: formation of mono- and bi-nuclear copper(II) complexes with a binucleating hexaaza macrocycle in slightly acidic solutions†

Manuel G. Basallote,* Joaquín Durán, M. Jesús Fernández-Trujillo, M. Angeles Máñez and Bruno Szpoganicz

Departamento de Ciencia de los Materiales e Ingeniería Metalúrgica y Química Inorgánica, Universidad de Cádiz, Facultad de Ciencias, Apartado 40, Puerto Real, 11510 Cádiz, Spain

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Reaction of the macrocycle 3,6,9,17,20,23-hexaazatricyclo[23.3.1.1^{11,15}]triaconta-1(29),11(30),12,14,25,27-hexaene (L) with Cu^{II} in borate–mannitol buffers at pH close to 5 occurred with two kinetically separated steps that correspond to the formation of mono- and bi-nuclear complexes. The rate constants for the two steps only differ by a factor of 7 and indicate that both metal ions are co-ordinated without significant interaction between them. However, the pH dependence of the rate constants suggests some kind of interaction of the buffering agent with the highly protonated forms of the macrocycle. Since this interaction is difficult to analyse due to the complex composition of the buffer, the kinetics of reaction have also been studied in the presence of the simpler acetate buffers. Complex formation also occurs in this case with two separate absorbance changes for the entry of both metal ions into the cavity of the macrocycle, and the analysis of the kinetic data is facilitated by a previous equilibrium study on the formation of ligand–acetate and Cu–L–acetate complexes. The stability constants of the species H_xL^{x+} ($x = 4$, 5 or 6), $H_3CuL(ac)^{4+}$, $H_2CuL(ac)^{3+}$, $Cu_2L(ac)^{3+}$ and $Cu_2L(ac)(OH)^{2+}$ have been determined from potentiometric titrations and their values used to separate the contributions from the different reaction pathways to the rate constants for complex formation. Although there are several contributions to the rate constant for the formation of mono- and bi-nuclear complexes, the analysis of kinetic data indicates that complex formation occurs essentially through reaction between those species that minimise the electrostatic repulsion. The rate constants for the reaction between Cu^{2+} and H_4L^{4+} (4.0×10^4 dm^3 mol^{-1} s^{-1}) and between $Cu(ac)_2$ and $H_2CuL(ac)^{3+}$ (1.2×10^4 dm^3 mol^{-1} s^{-1}) are close to each other and also close to those previously reported for the formation of copper(II) complexes with the tetraprotonated forms of linear acyclic polyamines, which suggests that the macrocycle is flexible enough to make rapid any reorganisation step required for complex formation.

Binuclear metal complexes of polyaza macrocycles have been used in the past years to study several aspects related to bioinorganic processes, such as the formation of dioxygen complexes,[1] the catalysed oxidation of organic substrates[2] or the hydrolysis of capped monoribonucleotides.[3] In most cases these processes are not catalysed by the analogous mononuclear complexes, or the catalytic activity of binuclear complexes is clearly higher. There are a large number of metalloenzymes that require the presence of two close metal ions to carry out their biological function[4] and, although better models can be found for every specific enzyme, metal complexes of binucleating macrocyclic ligands can be used as general models to understand the reactivity changes caused by the proximity of both metal centres. In addition, changing the structure of the macrocycle, which can be done in many ways, can conveniently modify any possible effect. For example, the distance between the metal ions and the co-ordination environment about them can be changed by varying the size of the macrocycle, by changing the nature of the donor atoms or by introducing external ligands able to act as bridges between the metal centres. We are especially interested in analysing the kinetic effects of these changes. It is expected that both metal ions behave as isolated centres for the case of complexes with a large macrocyclic cavity. However, as the size of the macrocycle is reduced and bridging ligands are introduced, it is reasonable to think that some kind of interaction between the metal ions will appear and this should result in an acceleration or retardation of their reactions. Probably the simplest of these reactions is complex formation, which requires the successive incorporation of two ions into the cavity of the macrocycle. Electrostatic repulsion between both ions can lead to a decrease in the rate of co-ordination of the second one, although this effect can be partially or totally compensated by the presence of negatively charged bridging ligands. We reported in a previous paper[5] the kinetics of formation of mono- and bi-nuclear copper(II) complexes with the macrocycle 3,6,9,17,20,23-hexaazatricyclo-[23.3.1.1^{11,15}]triaconta-1(29), 11(30),12,14,25,27-hexaene (L) under extremely basic conditions. In that case the ligand is completely deprotonated and reacts with $Cu(OH)_3^-$ and $Cu(OH)_4^{2-}$ to form mono- and bi-nuclear hydroxo-complexes,[6] but the co-ordination of the second ion is rapid,[5] probably as a consequence of the high tendency of these compounds to form hydroxo-bridged complexes.[7] These findings can not be extrapolated to complex formation at moderate pH because under these conditions Cu^{II} does not form hydroxo-complexes and the macrocycle exists as a mixture of several protonated forms.[5,6] In this paper we report a kinetic study of Cu^{II}–L complex formation in slightly acidic solutions. Although the kinetics is now complicated by the presence of a large number of species, the results indicate that co-ordination of the second metal ion occurs at a rate similar to that of the first one. Complex formation occurs essentially through those pathways that minimise electrostatic interaction between the reagents, and the macrocycle seems to be flexible enough to undergo rapidly all the

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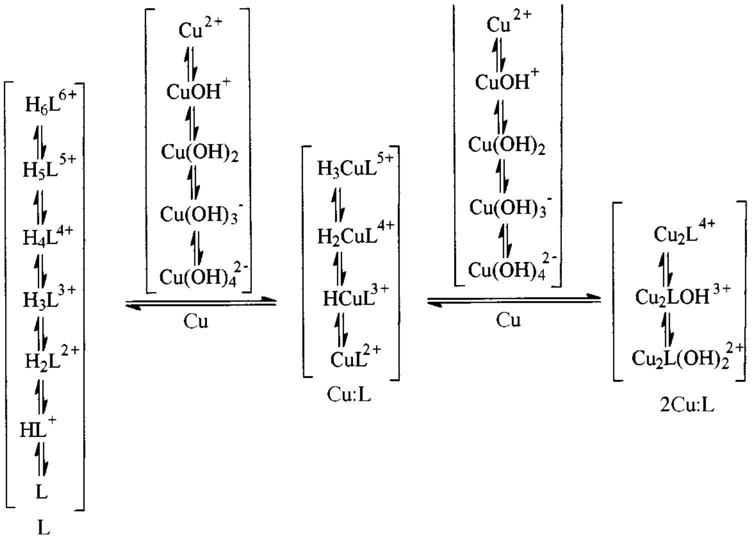
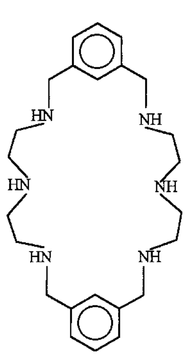
† *Supplementary data available*: derivation of rate equations, observed rate constants. For electronic access see http://www.rsc.org/suppdata/dt/1999/1093/, otherwise available from BLDSC (No. SUP 57502, 8 pp.) or the RSC Library. See Instructions for Authors, 1999, Issue 1 (http://www.rsc.org/dalton).



Scheme 1

reorganisation steps required for the formation of the different mono- and bi-nuclear complexes.



L

Results and discussion

Macrocycle L and other symmetrical hexaaza macrocycles with two N_3-donor subunits form a large variety of mono- and binuclear metal complexes depending on the pH and the total concentrations of ligand and metal ion. Equilibrium studies[6,8] indicate that the species included in Scheme 1 constitute a good equilibrium model for the case of copper(II) complexes. In mononuclear complexes the metal ion is co-ordinated to one N_3 subunit, and the other three nitrogen atoms can be protonated in acidic solutions to form $H_xCuL^{(2+x)+}$ complexes ($x = 0$, 1, 2 or 3). Although formation of mononuclear hydroxo-complexes has been observed for related ligands, these species are not formed for the case of L.[6] However, hydroxo-bridged species dominate the chemistry of binuclear complexes because the existence of one or more bridges with negative charge reduces the electrostatic repulsion between both metal ions and so Cu_2L^{4+} converts easily into $Cu_2L(OH)_x^{(4-x)+}$ ($x = 1$ or 2).[6] The relative concentrations of the different species in Scheme 1 depend on the actual conditions used to prepare the solutions. For the case of solutions containing L and an excess of Cu^{II}, formation of binuclear complexes is favoured and these species will be formed according to eqns. (1) and (2), where Cu, L,

$$Cu + L \rightleftharpoons Cu{:}L \qquad (1)$$

$$Cu{:}L + Cu \rightleftharpoons 2Cu{:}L \qquad (2)$$

Cu:L and 2Cu:L actually represent a mixture of the different species included in Scheme 1.

The large number of species involved makes too complicated a general analysis of kinetic data for the processes represented by eqns. (1) and (2). The problem can be simplified by working at selected pH ranges, which reduces the number of species formed at significant concentrations. For example, kinetic data at very high pH could be easily analysed[5] because under these conditions the ligand is completely deprotonated and Cu only exists as $Cu(OH)_3^-$ and $Cu(OH)_4^{2-}$. Hydrolysis of the metal ion is suppressed in strongly acidic solutions but formation of metal complexes is not favoured under these conditions. At intermediate pH values there is a larger number of species involved in eqns. (1) and (2), and the problem can be only partially simplified by working in a narrow pH range. Since formation of Cu:L and 2Cu:L complexes releases H^+ from H_xL^{x+}, it is necessary to carry out kinetic studies in buffered solutions, but the buffer to be used must not form copper(II) complexes because this would require the addition of more species to the equilibrium model in Scheme 1. It has been reported[9,10] that borate–mannitol buffers can be used safely to study the kinetics of formation of copper(II) complexes at pH values down to *ca.* 5 and so we initially used this buffer to carry out the kinetic experiments.

Kinetics of complex formation in borate–mannitol buffers

The kinetics of formation of copper(II) complexes with the macrocycle L was studied at 25.0 °C in the presence of 1.0 mol dm^{-3} KNO_3. The pH was fixed at values close to 5 by using borate–mannitol buffers, and the absorbance changes were recorded with a stopped-flow instrument. Preliminary experiments using a diode-array detector revealed that the maximum absorbance changes occur at 640 nm and so this wavelength was selected for the kinetic runs.

For experiments in the presence of Cu^{II} in excess, mono- and bi-nuclear complexes are formed according to eqns. (1) and (2) and kinetic traces can be fitted by two consecutive exponentials to obtain the rate constants k_{1obs} and k_{2obs} (SUP 57502). Although consecutive exponentials can have a number of different interpretations, the values of both constants change linearly with the total concentration of Cu^{II}, which strongly suggests that they correspond to the successive co-ordination of two metal ions, *i.e.* to the processes represented by eqns. (1) and (2). The values so derived for the second order rate constants corresponding to formation of mononuclear (k_1) and binuclear (k_2) complexes are included in Table 1, and they only show small changes with the pH. The quotient k_1/k_2 is always close to 7 and suggests that any reorganisation step previous to co-ordination of the second metal ion is rapid.

When the kinetic experiments are carried out in the presence

Table 1 Second-order rate constants for the formation of copper(II) complexes of L in borate–mannitol buffers at 25.0 °C[a]

pH	$10^4 k_1$/ dm^3 mol^{-1} s^{-1}	$10^3 k_2$/ dm^3 mol^{-1} s^{-1}	k_1/k_2
Excess Cu^{II}			
4.43	1.13(5)	1.72(4)	6.6
4.70	1.13(7)	1.45(8)	7.8
4.81[b]	1.34(6)	2.0(1)	6.7
Excess L[c]			
4.49	1.29(4)		
4.70	1.86(2)		
4.91	2.96(2)		
5.23	5.64(6)		

[a] The numbers in parentheses represent the standard deviations in the last significant digits. [b] Higher pH could not be used for experiments with excess Cu^{II} because of precipitation. [c] The values of k_2 cannot be determined because binuclear complexes are not formed under these conditions.

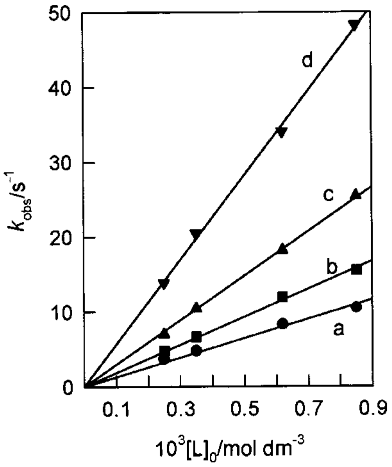


Fig. 1 Plots of the observed rate constant for the formation of mononuclear copper(II) complexes with macrocycle L at 25.0 °C in borate–mannitol buffers at different pH values: 4.49 (a), 4.70 (b), 4.91 (c) and 5.23 (d). The experiments were carried out under pseudo-first order conditions using $[Cu]_0 = 4.0 \times 10^{-5}$ mol dm^{-3} and excess ligand.

of ligand excess there is no significant formation of binuclear complexes and kinetic traces can be fitted satisfactorily by a single exponential. The values obtained for k_{1obs} are also included in SUP 57502, and the linear dependence with the ligand concentration is illustrated in Fig. 1. The values of the second order rate constant k_1 are included in Table 1, and their similarity with the values derived from experiments using excess metal confirm that they correspond to formation of mononuclear complexes [eqn. (1)]. However, the data show clearly now a marked dependence of k_1 with the pH, which must be interpreted in terms of a different reactivity of the species present in the reaction medium. As Cu^{II} exists only as Cu^{2+} under the conditions of the kinetic experiments,[9,10] any pH dependence of k_1 must be related to a different reactivity of the protonated forms of the ligand. The protonation constants of L indicate that H_4L^{4+} is the major form at pH close to 5, although minor amounts of H_5L^{5+} and H_6L^{6+} are also formed.[6] It is expected from electrostatic considerations that H_xL^{x+} species become less reactive as x increases, and this should lead to an increase of k_1 with pH. However, as H_4L^{4+} is the major species under all the conditions used and its concentration only increases slightly in the pH range from 4.49 to 5.23, the increase of k_1 should be much smaller than that observed experimentally. Thus, the changes in Table 1 can not be interpreted in terms of a different reactivity of the species included in Scheme 1, and the inert role of the buffer must be questioned. A possible explanation of kinetic data could be that the highly charged H_xL^{x+} species form stable complexes with borate anions, and this leads to major changes in the species distribution, in such a way that the concentration of the most reactive form increases significantly in the pH range considered. This effect would be more important in the experiments using ligand excess, and this would explain the difference between the values of k_{obs} derived from experiments using metal and ligand excess. The possibility of buffer complexation is supported by some literature reports showing that highly protonated forms of polyazamacrocycles form stable complexes with several anions.[2a,8c,11] Stability data corresponding to the formation of these borate complexes must be determined in order to test this hypothesis, but this is extremely difficult because of the complex nature of the borate–mannitol buffers. Nevertheless, kinetic data in Table 1 indicate that co-ordination of two copper(II) ions to macrocycle L at pH close to 5 occurs in two separate kinetic steps with comparable rate constants, and a detailed kinetic analysis could lead to a better understanding of the factors controlling the rate of these processes. As the analysis of kinetic data requires the knowledge of the equilibrium constants for the formation of complexes between the anion of the buffer and the protonated forms of the ligand, we decided to carry out the kinetic measurements in acetate buffers. In this case, the nature of the buffering agent is simpler and its acid–base behaviour is well known, which simplifies the determination of the stability constants for any possible H_xL^{x+}–acetate complex.

Equilibrium data for the Cu^{II}–L–acetate system

The equilibrium constants for ligand protonation and formation of metal complexes have been determined by the standard potentiometric procedures frequently used for hexaaza macrocycles,[1a,6–8] and the experimental and computational details are given in the Experimental section. Martell and coworkers[6] had previously determined the values corresponding to the six protonation steps of macrocycle L as well as the formation constants for mono- and bi-nuclear copper(II) complexes. We repeated these determinations before facing up to the problem of acetate complexation, and the values so obtained are included in Table 2 and compared with those previously reported.[6] There is in general an excellent agreement between both sets of data, with minor differences that can easily be explained in terms of experimental and fitting errors, except that we have included a H_3CuL^{5+} species not reported previously. This species should contain a copper(II) ion co-ordinated to one N_3 subunit of the macrocycle and so the constant in Table 2 should correspond to protonation of the last nitrogen atom, with a value that must be close to the sixth protonation constant for the ligand (3.34 *vs.* 3.37). This species is only formed at the most acidic points of the ligand titration, and a possible reason for the different results obtained by the two groups could be that the samples of the ligand used contained different amounts of HBr and titrations started from a different pH. According to our results, H_3CuL^{5+} only reaches a maximum concentration of 16% during the titrations and so its formation constant is not very well defined. However, titration data in the presence of acetate also suggest the existence of a triprotonated mononuclear species (see below) and we consider that the equilibrium model is more consistent with the inclusion of H_3CuL^{5+}. Martell and co-workers[13] also proposed the formation of a similar species for the case of a closely related macrocycle that only differs from L in the existence of two dipropylenetriamine sub-units. In that case the formation constant for the H_3CuL^{5+} species is 6.27, also close to the sixth protonation constant of the ligand (6.59).[13]

The analysis of titration curves of the ligand in the presence of added acetate (ac) requires the inclusion of $H_xL(ac)^{(x-1)+}$ species ($x = 4$, 5 or 6) in the equilibrium model. The constants

Table 2 Logarithms of equilibrium constants for the system Cu^{II}–L–acetate (25.0 °C, 0.1 mol dm^{-3} KNO$_3$)

K	Equilibrium quotient	log K^a
K_{HL}	[HL]/[L][H]	9.58 (9.49)
K_{H_2L}	[H$_2$L]/[HL][H]	8.79 (8.73)
K_{H_3L}	[H$_3$L]/[H$_2$L][H]	8.08 (8.03)
K_{H_4L}	[H$_4$L]/[H$_3$L][H]	7.34 (7.29)
K_{H_5L}	[H$_5$L]/[H$_4$L][H]	3.70 (3.64)
K_{H_6L}	[H$_6$L]/[H$_5$L][H]	3.37 (3.45)
$K_{H_4L(ac)}$	[H$_4$L(ac)]/[H$_4$L][ac]	1.42
$K_{H_5L(ac)}$	[H$_5$L(ac)]/[H$_5$L][ac]	1.88
$K_{H_6L(ac)}$	[H$_6$L(ac)]/[H$_6$L][ac]	2.27
K_{Hac}	[Hac]/[ac][H]	(4.56)
$K_{Cu(ac)}$	[Cu(ac)]/[Cu][ac]	(1.82)
$K_{Cu(ac)_2}$	[Cu(ac)$_2$]/[Cu(ac)][ac]	(1.0)
$K_{Cu(ac)_3}$	[Cu(ac)$_3$]/[Cu(ac)$_2$][ac]	(0.5)
K_{CuL}	[CuL]/[Cu][L]	13.86 (13.79)
K_{HCuL}	[HCuL]/[CuL][H]	8.25 (8.69)
K_{H_2CuL}	[H$_2$CuL]/HCuL][H]	7.64 (7.32)
K_{H_3CuL}	[H$_3$CuL]/[H$_2$CuL][H]	3.34
K_{Cu_2L}	[Cu$_2$L]/[CuL][Cu]	10.20 (9.68)
$K_{Cu_2L(OH)}$	[Cu$_2$L(OH)][H]/[Cu$_2$L]	−7.48 (−7.26)
$K_{Cu_2L(OH)_2}$	[Cu$_2$L(OH)$_2$][H]/[Cu$_2$L(OH)]	−8.70 (−8.40)
$K_{H_2CuL(ac)}$	[H$_2$CuL(ac)]/[H$_2$CuL][ac]	2.45
$K_{H_3CuL(ac)}$	[H$_3$CuL(ac)]/[H$_3$CuL][ac]	3.39
$K_{Cu_2L(ac)}$	[Cu$_2$L(ac)]/[Cu$_2$L][ac]	3.42
$K_{Cu_2L(ac)(OH)}$	[Cu$_2$L(ac)(OH)]/[Cu$_2$L(OH)][ac]	1.58

[a] Values from refs. 6 and 12 are shown in parentheses.

included in Table 2 indicate a higher stability of these species as *x* increases, which is reasonable in terms of electrostatic considerations. The same reason can be invoked to explain the lower stability of these acetate species with respect to those formed by related hexaaza macrocycles and dicarboxylate anions,[2a,13] that typically have log *K* values in the range 3.5–5.

Table 2 also includes mono- and bi-nuclear Cu^{II}–L complexes containing acetate, whose existence is based on the analysis of the titration curves for solutions containing Cu, L and acetate at different molar ratios. It is interesting that the stability constants for the formation of H$_3$CuL(ac)$^{4+}$ and H$_2$CuL(ac)$^{3+}$ are higher than that corresponding to Cu(ac)$^+$, which suggests that these species are stabilised by some additional interaction of co-ordinated acetate with the protonated nitrogen atoms. Protonation of H$_2$CuL(ac)$^{3+}$ to form H$_3$CuL(ac)$^{4+}$ occurs with a constant (log $K = 4.28$) higher than that corresponding to protonation of H$_2$CuL^{4+} (log $K = 3.34$), consistent with decreased Coulombic repulsion because of the charge changes. The value of 3.42 for the formation of Cu$_2$L(ac)$^{3+}$ from Cu$_2$L^{4+} is also high for a single Cu–ac bond and suggests the formation of an acetato bridge between both metal centres. On the contrary, the value found for Cu$_2$L(ac)(OH)$^{2+}$ is lower (1.58) and consistent with a terminal acetate. Nevertheless, the possibility of a double bridge can not be ruled out because there are several well characterised binuclear copper(II) complexes with a double μ-OH-μ-acetato bridge.[14] Although the number of acetate-containing species detected in the potentiometric studies is not very high, under certain conditions they become the major species in solution. For example, Fig. 2 includes the species distribution diagram for solutions containing Cu, L and acetate in 2 : 1 : 2 molar ratios, and it shows that Cu$_2$L(ac)$^{3+}$ is the major species over a wide range of pH values. On the contrary, Cu$_2$L(ac)(OH)$^{2+}$ only reaches a maximum of 5% during the titration and so its constant is not well defined. Mononuclear acetate-containing species only reach maximum percentages lower than 20% under these conditions but they are formed at higher concentrations in titrations containing one equivalent of Cu^{II} per equivalent of ligand. The amount of mono- and bi-nuclear complexes without co-ordinated acetate is important at the concentrations of Fig. 2 but these species are formed at a much lower extent under the conditions used in the kinetic experiments.

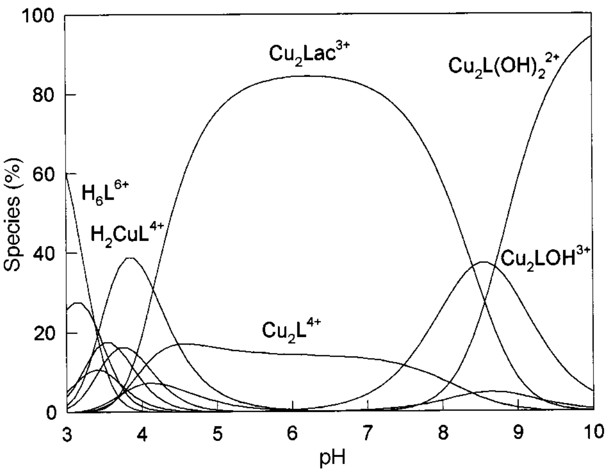


Fig. 2 Species distribution curves showing the species formed in solutions containing the macrocycle L, Cu^{II} and acetate at molar ratios 1 : 2 : 2 ([L]$_0$ = 2 × 10^{-3} mol dm^{-3}). The curves corresponding to the major species have been labelled but the Figure also includes some unlabelled curves that correspond to the following species (the maximum percentage and the pH at which this is reached are given in parentheses): H$_5$L^{5+} (28, pH 3.1), H$_4$L^{4+} (11, pH 3.4), H$_3$CuL^{5+} (18, pH 3.5), H$_3$CuL(ac)$^{4+}$ (16, pH 3.8), H$_2$CuL(ac)$^{3+}$ (7, pH 4.1), and Cu$_2$L(ac)(OH)$^{2+}$ (5%, pH 8.6).

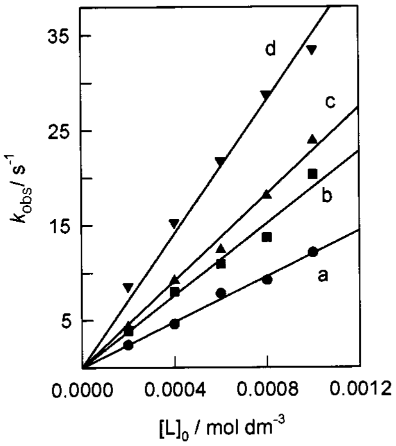


Fig. 3 Plots of the observed rate constant for the formation of mononuclear copper(II) complexes with macrocycle L at 25.0 °C in acetate buffers at different pH values: 3.90 (a), 4.16 (b), 4.40 (c) and 4.96 (d). The experiments were carried out under pseudo-first-order conditions using [Cu]$_0$ = 4.0 × 10^{-5} mol dm^{-3}, [acetate]$_0$ = 0.05 mol dm^{-3}, and ligand excess.

Kinetics of complex formation in acetate buffers

Ligand excess. According to the equilibrium data in the previous section, only mononuclear Cu : L complexes will be formed upon reaction of Cu^{II} with an excess of ligand in the presence of an acetate buffer. The reaction occurs within the stopped-flow timescale and it shows a single kinetic step that can be well fitted by a single exponential to obtain the rate constants k_{obs} (SUP 57502). The linear dependence of k_{1obs} on the ligand concentration is illustrated in Fig. 3, and the values of the second order rate constant k_1 are included in Table 3. These data are of the same order of magnitude as those found in borate–mannitol buffers, and they also indicate that the rate of formation of mononuclear complexes increases with pH, although the knowledge of the equilibrium constants in Table 2 facilitates now a more detailed analysis of the kinetic data.

Table 3 Rate constants for the formation of mononuclear copper(II) complexes of L in acetate buffers at 25 °C. These results correspond to experiments in the presence of 0.05 mol dm^{-3} acetate and ligand excess[a]

pH	$10^{-4}\,k_1$/ dm^3 mol^{-1} s^{-1}	10^2 [ac$^-$]/ mol dm^{-3}
3.90	1.20(2)	0.89
4.16	1.90(7)	1.42
4.40	2.29(6)	2.03
4.96	3.52(9)	3.55

[a] The numbers in parentheses represent the standard deviations in the significant digits. The values of k_1 correspond to formation of mononuclear complexes and were derived from experiments under pseudo-first-order conditions using $[\mathrm{Cu}]_0 = 4.0 \times 10^{-5}$ mol dm^{-3} and ligand excess.

Formation of mononuclear complexes can be represented in a simplified way by eqn. (1) but the reagents actually exist as a mixture of several species. In any case, the concentration of all the species in the reaction medium before complex formation can be calculated by running the program SPE[15] for all the conditions used in the kinetic experiments but without including in the input file the Cu : L and 2Cu : L complexes. These calculations indicate that Cu exists as a mixture of Cu^{2+} (60–24%), $Cu(ac)^+$ (36–55%) and $Cu(ac)_2$ (3–19%), whereas L exists as a complex mixture of six protonated species, H_xL^{x+} and $H_xL(ac)^{(x-1)+}$ ($x = 4$, 5 or 6), the major species being those with $x = 4$, although none represents more than 50% of the total ligand under the conditions used in the kinetic experiments. Some of these ligand species are expected to be poorly reactive, even so there are many possible reaction pathways for the formation of mononuclear complexes. The pH dependence of k_1 suggests the existence of a reactive form with a pK_a close to 4–5, probably H_4L^{4+} or $H_4L(ac)^{3+}$. From electrostatic considerations these are expected to be the most reactive forms of the ligand, but the reactive form of Cu^{II} is not so evident. Although there are literature reports showing that all the copper–acetate complexes react at similar rates,[17] a different reactivity of complexed and uncomplexed Cu^{II} has also been observed in some cases.[18,19] In any case, an attempt can be made to separate the contributions of the different pathways by calculating the product $k_1D_{Cu}D_L$,where D_{Cu} and D_L are defined in eqns. (3) and (4).

$$D_{Cu} = 1 + K_{Cuac}[ac^-] + K_{Cuac_2}K_{Cuac}[ac^-]^2 + K_{Cuac_3}K_{Cuac_2}K_{Cuac}[ac^-]^3 \quad (3)$$

$$D_L = 1 + K_{H_5L}[H^+] + K_{H_6L}K_{H_5L}[H^+]^2 \quad (4)$$

The acetate concentration can be calculated with program SPE and it is essentially constant within a series of kinetic experiments at constant pH; the values are given in Table 3. The plot in Fig. 4 shows that $k_1D_{Cu}D_L$ changes linearly with $[ac^-]^2$, and a least squares fit using eqn. (5) yields the values of

$$k_1D_{Cu}D_L = a + b[ac^-]^2 \quad (5)$$

$a = (4.0 \pm 0.4) \times 10^4$ dm^3 mol^{-1} s^{-1} and $b = (2.25 \pm 0.06) \times 10^8$ mol^{-1} dm^{-3} s^{-1}. The inclusion of additional terms does not lead to a better fit, although it is possible that they appear for data corresponding to other reaction conditions.

The existence of a non-zero intercept indicates a significant contribution from reaction between Cu^{2+} and H_4L^{4+}, with a rate constant of 4.0×10^4 dm^3 mol^{-1} s^{-1}. The possibility that the less protonated forms of the ligand are highly reactive and responsible for the a term can be ruled out because this would lead to unreasonably high values of the second order rate constant. For example, if a is ascribed to reaction of H_3L^{3+} with Cu^{2+} the rate constant would be in the range 10^7–10^8 dm^3 mol^{-1} s^{-1}, close to that found for reaction with neutral NH_3 and much larger than the values for reaction of Cu^{2+} with less protonated forms of other polyamines and azamacrocycles.[10,19] On the other hand, the value derived for reaction between Cu^{2+} and H_4L^{4+} compares well with those previously reported for the formation of copper(II) complexes with the tetraprotonated form of the less sterically demanding linear tetraethylenepentaamine [(1.4–3.3) × 10^4 dm^3 mol^{-1} s^{-1}],[10,19] which suggests that the rate determining step is probably the same in the two cases and that reorganisation of the macrocycle does not cause any significant kinetic difference. The absence of additional kinetic steps in the formation of mononuclear Cu^{II}–L–ac complexes suggests that the metal ion is accommodated rapidly into the cavity of the ligand, *i.e.* the macrocycle is flexible enough to undergo rapidly any reorganisation required to form the metal complexes.

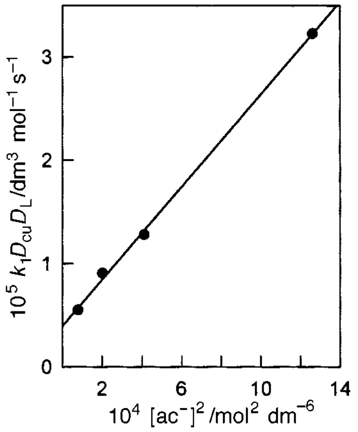


Fig. 4 Plot showing the linear dependence of the product $k_1D_{Cu}D_L$ with $[ac^-]^2$ (see text for the definition of k_1, D_{Cu} and D_L). The data correspond to experiments in acetate buffers using an excess of ligand.

There is some ambiguity about the meaning of the b term in eqn. (5) because it corresponds to the sum of contributions from two different reaction pathways. This is a very common problem in the kinetics of reactions involving pre-equilibrium for any of the reagents,[20] and this ambiguity means it is only possible to obtain upper limits for the rate constants corresponding to each of the possible pathways. In this case, the values of b and the equilibrium constants in Table 2 lead to limits of 1.3×10^5 dm^3 mol^{-1} s^{-1} for the reaction between $Cu(ac)^+$ and $H_4L(ac)^{3+}$, and 3.4×10^5 dm^3 mol^{-1} s^{-1} for reaction between $Cu(ac)_2$ and H_4L^{4+}.

Metal excess. Equilibrium considerations indicate that formation of binuclear species is favoured in the presence of an excess of the metal ion. Experimental conditions were selected in such a way that formation of mononuclear species occurs within the mixing time of the stopped-flow instrument, and the kinetic traces obtained show absorbance changes that can be fitted by a single exponential to give the rate constants k_{obs} (SUP 57502). Under these conditions, the formation of mononuclear complexes simply causes a change in the value of the initial absorbance and the values of k_{obs} refer to the conversion of mononuclear to binuclear complexes [eqn. (2)], and its dependence on the total concentration of Cu^{II} is illustrated in Fig. 5.

This dependence can be represented by eqn. (6), which is

$$k_{obs} = k_2[Cu]_0 + k_{-s} \quad (6)$$

frequently observed in the kinetics of complex formation under reversible conditions and is usually interpreted assuming that k_2 and k_{-2} correspond to the rate constants for complex formation and decomposition, respectively.[21] The values so derived for k_2 and k_{-2} are included in Table 4 and show that both of them depend on the concentration of H^+ and acetate, although the actual dependence is not easy to determine. The minimum

Table 4 Rate constants for the formation of binuclear copper(II) complexes of L in acetate buffers at 25 °C. These results correspond to experiments in the presence of Cu^{II} [a]

	$[MeCO_2^-] = 0.05$ mol dm^{-3}		$[MeCO_2^-] = 0.10$ mol dm^{-3}	
pH	$10^3 k_2/$ dm^3 mol^{-1} s^{-1}	k_{-2}/s^{-1}	$10^3 k_2/$ dm^3 mol^{-1} s^{-1}	k_{-2}/s^{-1}
3.66	0.35(2)	9.4(6)	0.21(1)	15.2(4)
3.90	0.62(4)	8.3(1)	0.76(8)	8(3)
4.16	1.10(3)	1.4(9)	1.30(7)	5(2)
4.40	1.37(9)	4(3)	2.1(2)	8(6)

[a] The numbers in parentheses represent the standard deviations in the last significant digits. The values of k_2 and k_{-2} correspond to formation and decomposition of the binuclear complexes, respectively. They were derived from experiments using $[L]_0 = 1.0 \times 10^{-3}$ mol dm^{-3} and excess Cu^{II}. Under these conditions, formation of the mononuclear complexes is rapid, and the values of k_{obs} correspond to their conversion into the binuclear complexes.

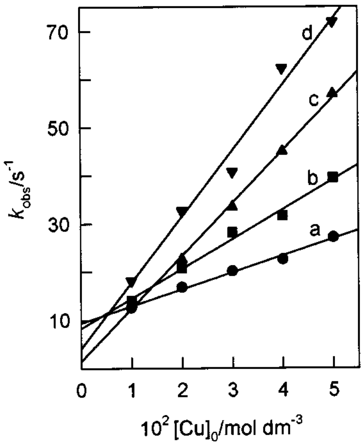


Fig. 5 Plots of the observed rate constant for the formation of binuclear copper(II) complexes with macrocycle L at 25.0 °C in acetate buffers at different pH values: 3.66 (a), 3.90 (b), 4.16 (c) and 4.40 (d). The experiments were carried out under pseudo-first-order conditions using $[L]_0 = 1.0 \times 10^{-3}$ mol dm^{-3}, $[acetate]_0 = 0.05$ mol dm^{-3}, and Cu^{II} in excess. Under these conditions, formation of the mononuclear complexes is rapid and the values of k_{obs} correspond to their conversion into the binuclear complexes.

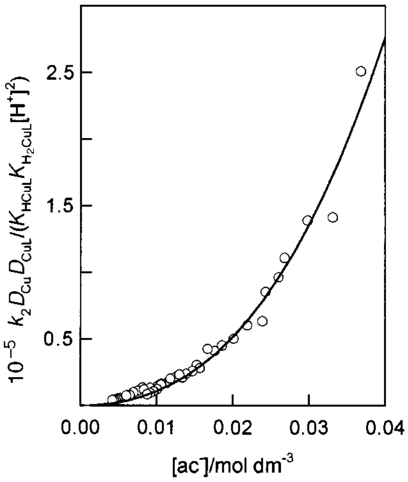


Fig. 6 Plot showing the dependence on the acetate concentration of the quotient $k_2 D_{Cu} D_{CuL}/(K_{HCuL} K_{H_2CuL}[H^+]^2)$ (see text for the definition of the different terms). The points correspond to experiments in acetate buffers using an excess of Cu^{II}, and the line represents the best fit of the data using eqn. (9) in the text.

observed for k_{-2} at pH 4.16 is not easy to understand and is probably related, at least in part, to the errors associated with its determination. However, it will be shown below that this minimum is replaced by a consistent decrease of k_{-2} with increasing pH when the data are re-evaluated considering the changes in acetate concentration. The species distribution curves corresponding to the conditions used in the kinetic experiments indicate that conversion into binuclear complexes is not complete, and the final reaction product is an equilibrium mixture of mono- and bi-nuclear complexes. Thus, only 30% of the ligand is converted into binuclear complexes at pH 3.66 when $[Cu]_0 = 0.01$ mol dm^{-3}, and the conversion only increases to 72% when $[Cu]_0 = 0.05$ mol dm^{-3}. These percentages increase at higher pH but there is in all cases a certain amount of mononuclear complexes, which confirms that the kinetic data must be interpreted in terms of a reversible equilibrium. The binuclear complexes formed exist mainly as $Cu_2L(ac)^{3+}$, with minor amounts of Cu_2L^{4+}.

As for the case of experiments in the presence of ligand excess, the analysis of kinetic data requires the knowledge of the concentrations of free acetate and H^+, and the equilibrium constants of the species involved. However, a careful inspection of the equilibrium data reveals that the acetate concentration changes within a series of kinetic data at constant pH, which indicates that Cu and Cu : L in eqn. (2) exist as a mixture of varying composition and suggests that the fit of kinetic data using eqn. (6) is probably accidental. For this reason, we decided to use the equilibrium data to carry out a more reliable separation of the k_{obs} values into their k_2 and k_{-2} components. The derivation of eqns. (7) and (8) is given in SUP, which also

$$k_{-2} = \frac{k_{obs}}{1 + (K_{Cu_2L}\, D_{Cu_2L}[Cu]_0/D_{CuL}D_{Cu})} \qquad (7)$$

$$k_2 = (k_{obs} - k_{-2})/[Cu]_0 \qquad (8)$$

includes expressions for D_{Cu}, D_{CuL} and D_{Cu_2L}. The values of k_2 and k_{-2} in SUP were obtained by using these equations and the concentrations of free acetate and H^+ calculated with program SPE. The major difference from the values in Table 4 consists in a significant decrease of k_{-2} for the experiments at higher pH values, which is not surprising because the conversion into binuclear complexes is almost complete under those conditions.

Once resolved the k_2 and k_{-2} components of k_{obs} it is necessary to determine the relative contributions of the different reaction pathways to these rate constants. Although the values in SUP 57502 seem to indicate a dependence on the concentrations of both H^+ and acetate, we did not attempt to analyse the different contributions to the rate constant for decomposition of binuclear complexes because of the large uncertainties in the k_{-2} values. In order to separate the different contributions to the rate constant for complex formation (k_2), the program SPE was used to determine the species distribution before formation of binuclear complexes. For this purpose, the binuclear species were not included in the input file and the program was run for all the different experimental conditions in SUP. The results showed that Cu^{II} exists mainly as Cu^{2+} and $Cu(ac)^+$, with minor amounts of $Cu(ac)_2$. On the other hand, mononuclear complexes exist as a mixture of $H_3CuL(ac)^{4+}$ and $H_2CuL(ac)^{3+}$, with minor amounts of H_3CuL^{5+} and H_2CuL^{4+}. There are thus up to 12 possible contributions to k_2 from reaction between the different Cu and Cu:L species. Nevertheless, the pH dependence of the rate constant suggests that the contributions from $H_3CuL(ac)^{4+}$ and H_3CuL^{5+} have little significance, which is not surprising because these species do not contain any nitrogen with a free electron pair. It can be demonstrated (SUP) that the dependence of the k_2 values on the concentration of acetate and H^+ is given by eqn. (9), where the

$$\frac{k_2 D_{CuL}\, D_{Cu}}{K_{HCuL}\, K_{H_2CuL}\, [H^+]^2} = \sum_{i=0}^{3} (a_i + b_i[H^+])[ac^-]^i \qquad (9)$$

Table 5 Summary of rate constants for the formation of mono- and bi-nuclear Cu^{II}–L complexes in acetate buffers at 25.0 °C [a]

Reagents	10^{-4} k/dm^3 mol^{-1} s^{-1}
Cu^{2+} + H_4L^{4+}	4.0(4)
$Cu(ac)^+$ + $H_4L(ac)^{3+}$	<12.9(3)
$Cu(ac)_2$ + H_4L^{4+}	<34.0(9)
$Cu(ac)_2$ + $H_2CuL(ac)^{3+}$	1.2(2)
$Cu(ac)_2$ + H_2CuL^{4+}	<13(2)
$Cu(ac)^+$ + $H_2Cu(ac)^{3+}$	<0.46(7)

[a] The numbers in parentheses represent the standard deviations in the last significant digits.

a_i and b_i terms include different rate and equilibrium constants. Although the theoretical equation predicts a complex behaviour, the fit of experimental data by eqn. (9) reveals a more simple dependence, with all the a_i and b_i terms being zero except $a_2 = (8.5 \pm 1.3) \times 10^7$ and $a_3 = (2.2 \pm 0.4) \times 10^9$ (see Fig. 6). The inclusion of additional terms does not result in any significant improvement in the quality of the fit.

The a_3 term corresponds to reaction between $H_2CuL(ac)^{3+}$ and $Cu(ac)_2$, and the expression given in SUP 57502 leads to a rate constant of 1.2×10^4 dm^3 mol^{-1} s^{-1} for the reaction between these two species. There is more ambiguity about the meaning of the a_2 term. Since it corresponds to the sum of two contributions, it is only possible to estimate upper limits for the rate constants corresponding to the reaction between H_2CuL^{4+} and $Cu(ac)_2$ (1.3×10^5 dm^3 mol^{-1} s^{-1}) or between $H_2CuL(ac)^{3+}$ and $Cu(ac)^+$ (4.6×10^3 dm^3 mol^{-1} s^{-1}). Although these numerical values must be taken with care because of the complex analysis of the kinetic data, they seem to indicate again that the kinetics of formation of these complexes is essentially controlled by electrostatic factors; the more effective reaction pathways being those that minimise repulsion between both reagents. Table 5 includes a summary of the rate constants determined in this work for the formation of mono- and bi-nuclear complexes of macrocycle L in acetate buffers. Unfortunately, because of the complex nature of the reaction mixtures formed in these systems it is not possible to make a direct comparison between the rate constants corresponding to co-ordination of both metal ions to the macrocycle. However, the values in Table 5 indicate that, despite the observed rate constants for co-ordination of both metal ions differ by *ca.* two orders of magnitude, this is a consequence of the changes in the concentration of the reacting species and the actual rate constants for co-ordination of the second metal ion are not very different from those corresponding to the first one, with the differences being easily explained in terms of electrostatic factors. Some of the values also suggest a certain decrease of the rate constant associated with the existence of one or more co-ordinated acetates, but the decrease is of less than one order of magnitude and can also include some compensation caused by favourable changes in the charge of the reagents. Although they were not analysed in detail, the data in borate–mannitol buffers also indicate close values for the rate constants of formation of mono- and bi-nuclear complexes. Thus, as a whole, kinetic data in this work suggest that the macrocycle L is flexible enough to allow the two N_3 subunits to behave almost independently. It would be the local charge on every subunit that controls the rate of reaction with the metal ions, with all the subsequent reorganisation steps being rapid. Further work is in progress to confirm these conclusions and to determine how these findings are modified by changes in the structure of the macrocycle.

Experimental

The ligand 3,6,9,17,20,23-hexaazatricyclo[23.3.1.1^{11,15}]triaconta-1(29),11(30),12,14, 25,27-hexaene was synthesized as the hexabromohydrate [$C_{24}H_{38}N_6$·6HBr(L·6HBr)] following the literature procedure.[6] All other reagents were obtained from Aldrich and used without further purification.

Kinetic experiments

The experiments were carried out at 25.0 °C with an Applied Photophysics stopped-flow instrument. The ionic strength of the solutions was adjusted to 1.0 mol dm^{-3} by adding the amount of KNO_3 required. All experiments were carried out under pseudo-first-order conditions of ligand or metal excess, and kinetic traces could be fitted satisfactorily by single or consecutive exponentials using the standard software of the stopped-flow instrument. The wavelength was selected at 640 nm because it corresponded to the maximum absorbance changes in preliminary spectral scanning experiments, that were also used to check the independence of the observed rate constants with changes in the concentration of the limiting reagent. Reported values of the rate constant correspond to the mean value for at least five determinations.

Equilibrium experiments

The KOH solutions used for the potentiometric titrations were obtained from Aldrich and titrated with potassium hydrogenphthalate. The pH readings were obtained with a Crison 2002 instrument provided with an Ingold combined electrode and calibrated according to the procedure recommended by Martell and Motekaitis.[15] Solutions of Cu^{II} were prepared from $Cu(NO_3)_2$·2.5 H_2O and titrated with EDTA using murexide as indicator.

The protonation constants of the ligand and the formation constants of ligand–acetate and Cu–L–acetate complexes were determined from several potentiometric titrations carried out at 25.0 °C under N_2 with solutions containing L, Cu^{II} and acetate at different molar ratios: 1:0:0, 1:1:0, 1:2:0, 1:0:1, 1:0:4, 1:1:1, 1:1:2, 1:2:1 and 1:2:2 (L:Cu:ac). The number of points measured was different for every titration, although there were typically 10 points for every neutralisation of a proton or every hydrolytic reaction. The ligand concentration was always in the range (1–2) × 10^{-3} mol dm^{-3}, and the concentrations of the other components were then adjusted to the molar ratios indicated above. The initial volume was close to 50.0 cm^3, and the ionic strength was adjusted to 0.1 mol dm^{-3} with KNO_3. Some additional titrations were made in the presence of 1.0 mol dm^{-3} KNO_3 but they were not included in the calculations because of precipitation under some conditions. The range of pH covered in the different titrations expands from *ca.* 3 to 11, and the data were analysed with the program BEST[16] assuming a pK_w value of 13.78. The species distribution curves were obtained with programs SPE and SPEPLOT.[16]

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References

1 (*a*) M. G. Basallote and A. E. Martell, *Inorg. Chem.*, 1988, **27**, 4219; (*b*) M. P. Ngwenya, D. Chen, A. E. Martell and J. Reibenspies, *Inorg. Chem.*, 1991, **30**, 2732; (*c*) M. Becker, S. Schindler and R. Van Eldik, *Inorg. Chem.*, 1994, **33**, 5370; (*d*) S. Mahapatra, S. Kaderli, A. Llobet, Y. Neuhold, T. Palanché, J. A. Halfen, V. G. Young, Jr., T. A. Kaden, L. Que, Jr., A. D. Zuberbüller and W. B. Tolman, *Inorg. Chem.*, 1997, **36**, 6343.

2 (*a*) R. J. Motekaitis and A. E. Martell, *Inorg. Chem.*, 1991, **30**, 694; (*b*) D. A. Rockcliffe and A. E. Martell, *Inorg. Chem.*, 1993, **32**, 3143.

3 K. P. McCue, D. A. Voss, Jr., C. Marks and J. R. Morrow, *J. Chem. Soc.*, *Dalton Trans.*, 1998, 2961.

4 J. M. Brown, L. Powers, B. Kincaid, J. A. Larrabee and T. G. Spiro, *J. Am. Chem. Soc.*, 1980, **102**, 4210; J. A. Tanier, E. D. Getzoff, K. M. Beem, J. S. Richardson and D. C. Richardson, *J. Mol. Biol.*, 1982, **106**, 181; G. L. Woolery, L. Powers, M. Winkler, E. J. Solomon and T. G. Spiro, *J. Am. Chem. Soc.*, 1984, **106**, 86; R. M. Fronko, J. F. Penner-Hahn and C. J. Bender, *J. Am. Chem. Soc.*, 1988, **110**, 7554.

5 M. J. Fernández-Trujillo, B. Szpoganicz, M. A. Máñez, L. T. Kist and M. G. Basallote, *Polyhedron*, 1996, **15**, 3511.

6 R. Menif, A. E. Martell, P. J. Squattrito and A. Clearfield, *Inorg. Chem.*, 1990, **29**, 4723.

7 R. J. Motekaitis, A. E. Martell, J. P. Lecompte and J. M. Lehn, *Inorg. Chem.*, 1983, **22**, 609; R. J. Motekaitis, A. E. Martell and I. Murase, *Inorg. Chem.*, 1986, **25**, 938.

8 (*a*) R. Menif, D. Chen and A. E. Martell, *Inorg. Chem.*, 1989, **28**, 4633; (*b*) R. Menif, J. Reibenspies and A. E. Martell, *Inorg. Chem.*, 1991, **30**, 3446; (*c*) R. J. Motekaitis and A. E. Martell, *Inorg. Chem.*, 1992, **31**, 5534.

9 D. W. Margerum, B. A. Zabin and D. L. Janes, *Inorg. Chem.*, 1966, **5**, 250.

10 D. B. Moss, C. T. Lin and D. B. Rorabacher, *J. Am. Chem. Soc.*, 1973, **95**, 5179.

11 E. Graf and J. M. Lehn, *J. Am. Chem. Soc.*, 1976, **98**, 6403; J. M. Lehn, E. Sonveaux and A. K. Willard, *J. Am. Chem. Soc.*, 1978, **100**, 4914; B. Dietrich, M. W. Hosseini, J. M. Lehn and R. B. Sessions, *J. Am. Chem. Soc.*, 1981, **103**, 1282; M. W. Hosseini and J. M. Lehn, *J. Am. Chem. Soc.*, 1982, **104**, 3525.

12 R. M. Smith, A. E. Martell and R. J. Motekaitis, NIST Critical Stability Constants of Metal Complexes Database, U.S. Dept of Commerce, Gaithesbourgh, 1993.

13 A. Llobet, J. Reibenspies and A. E. Martell, *Inorg. Chem.*, 1994, **33**, 5946.

14 C. J. Boxwell, R. Bhalla, L. Cronin, S. S. Turner and P. H. Walton, *J. Chem. Soc.*, *Dalton Trans.*, 1998, 2449.

15 A. E. Martell and R. J. Motekaitis, *Determination and use of stability constants*, VCH, New York, 1992.

16 S. P. Kasprzyk and R. G. Wilkins, *Inorg. Chem.*, 1988, **27**, 1834; M. Kodama and E. Kimura, *J. Chem. Soc.*, *Dalton Trans.*, 1977, 1473.

17 R. W. Hay and C. R. Clark, *J. Chem. Soc.*, *Dalton Trans.*, 1977, 1148.

18 R. E. Shepherd, G. M. Hodgson and D. W. Margerum, *Inorg. Chem.*, 1971, **10**, 989.

19 B. C. Westerby, K. L. Juntunen, G. H. Leggett, V. B. Pett, M. J. Koenigbauer, M. D. Purgett, M. J. Taschner, L. A. Ochrymowycz and D. B. Rorabacher, *Inorg. Chem.*, 1991, **30**, 2109.

20 R. G. Wilkins, *Kinetics and Mechanism of Reactions of Transition Metal Complexes*, 2nd edn., VCH, Weinheim, 1991, pp. 77–78.

21 V. B. Pett, G. H. Legget, T. H. Cooper, P. R. Reed, D. Situmeang, L. A. Ochrymowycz and D. B. Rorabacher, *Inorg. Chem.*, 1988, **27**, 2164; M. S. Chao and C. S. Chung, *Inorg. Chem.*, 1989, **28**, 686; K. Krylova, K. D. Jackson, J. A. Vroman, A. J. Grall, M. R. Snow, L. A. Ochrymowycz and D. B. Rorabacher, *Inorg. Chem.*, 1997, **36**, 6216.

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