Water-soluble Hexadentate Schiff-base Ligands as Sequestrating Agents for Iron(III) and Gallium(III)

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Complexes of Fe^{III} and Ga^{III} with hexadentate Schiff-base ligands have been characterised in aqueous solution. They were prepared by the condensation of salicylaldehydes, containing a sulphonate or trimethylammonium group, with polyamines in the presence of the metal ions. These complexes have stabilities, at physiological pH, similar to those of the hydroxamic acid siderophore complexes. The kinetics of the displacement of Fe^{III} from $[Fe^{III}(edta)]^-$ (edta = ethylenediamine-*N,N,N',N'*-tetra-acetate) by two of these ligands has been studied.

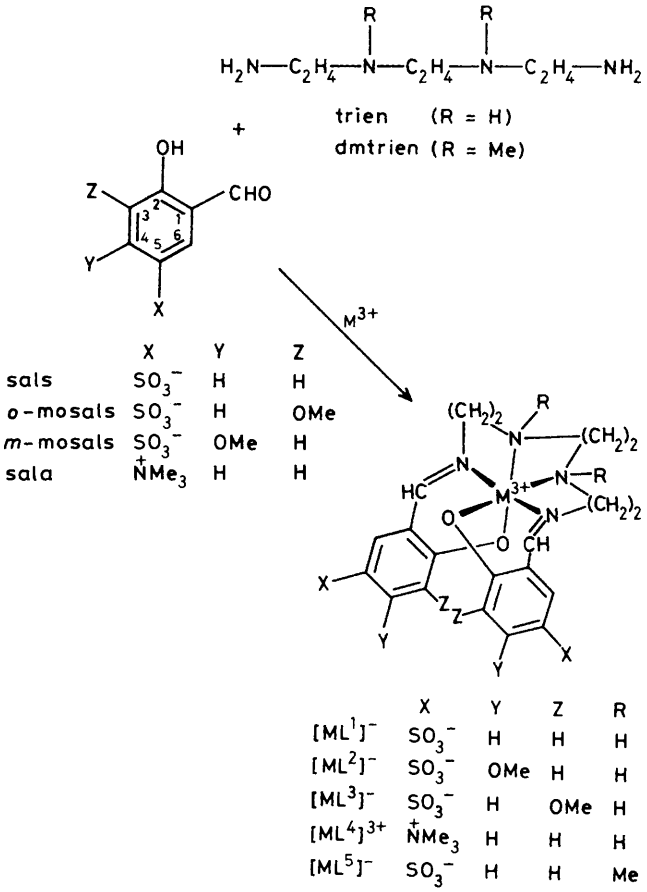
In recent years there has been considerable interest in siderophores, chelating agents for Fe^{III}, which are involved in the transport of iron in biological systems.[1] These complexes normally have six oxygens in an octahedral sphere about the Fe^{III}, with the most important classes containing hydroxamic acid (*e.g.* ferrichrome, ferrioxamine) or catechol groups (*e.g.* enterobactin). These natural siderophores, and synthetic analogues, have been extensively studied with respect to their use in iron overload treatment.[2] Currently there is also interest in stable Ga^{III} complexes for use as radiopharmaceuticals[3] and in ^{68}Ga positron emission tomography.[4] Due to the very similar ionic radii[5] and equal charge of the Fe^{III} and Ga^{III} ions one often observes similar co-ordination chemistry for the two ions and ligands with a high affinity for Fe^{III} tend also to have a high affinity for Ga^{III}.

We report some water-soluble hexadentate Schiff-base ligands and their complexes with Fe^{III} and Ga^{III} in aqueous solution. These complexes have stabilities which are similar to those formed by natural hydroxamic acid siderophores, and considerably greater than for those formed by ethylenediamine-*N,N,N',N'*-tetra-acetic acid (H_4edta) and diethylenetriamine-*N,N,N',N',N''*-penta-acetic acid (H_5dtpa), with Fe^{III} and Ga^{III}.

Although hexadentate Schiff bases have been studied over many years following their initial use by Dwyer *et al.*,[6] in the majority of cases the ligands and/or the metal complexes are water-insoluble. Recently salicylaldehyde-5-sulphonate (sals) has been used to form water-soluble tetradentate Schiff-base complexes with Co^{II},[7] Co^{III},[7] Ni^{II},[8,9] $V^{IV}O^{2+}$,[10] and V^{III}.[10] The new hexadentate Schiff-base ligands reported here are formed by the condensation of aromatic *o*-hydroxyaldehydes, incorporating a sulphonate group (*e.g.* sals) or a trimethylammonium group, with polyamines.

Since in aqueous solution the Schiff bases are partially hydrolysed to the starting materials their isolation was not attempted, but instead stoicheiometric amounts of the desired aldehyde and polyamine were used to generate the ligands *in situ*. Template reactions are thus, to some extent, involved in the formation of the complexes (see Schemes 1 and 2).

The kinetics of the displacement of edta from $[Fe^{III}(edta)]^-$ by the Schiff-base ligands was also studied.



Scheme 1. $M^{3+} = Fe^{3+}$

Experimental

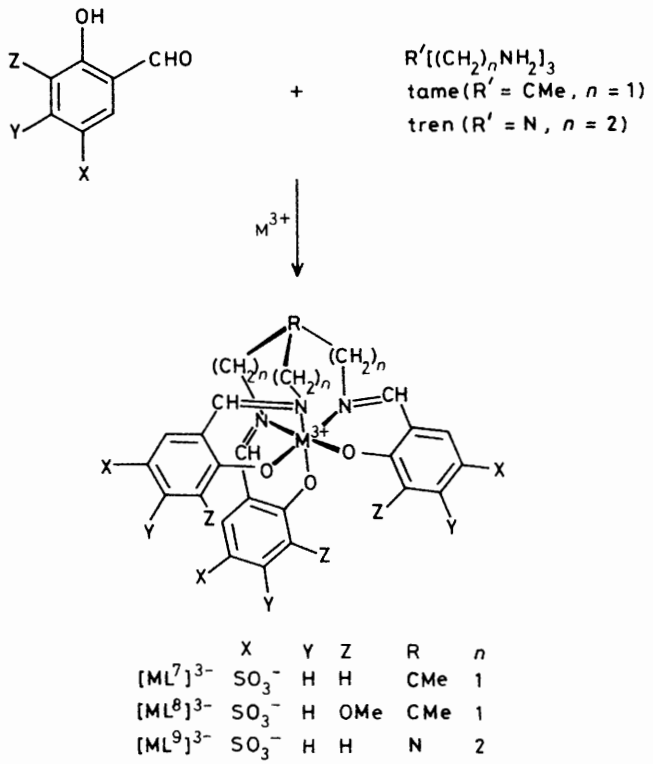
Materials and Synthesis.—The ligand sals was prepared as described previously[8] but dried at 110 °C *in vacuo* to produce the anhydrous salt (Found: C, 37.55; H, 2.20. $C_7H_5NaO_5S$ requires C, 37.50; H, 2.25%). The ligands *o*-mosals and *m*-mosals were prepared by the method for sals but starting with the appropriate methoxysalicylaldehyde and carrying out the sulphonation at 70 and 40 °C respectively. Yields: *o*-mosals, 60% (Found: C, 37.65; H, 2.70. $C_8H_7NaO_6S$ requires C, 37.80; H, 2.80%); *m*-mosals, 64% (Found: C, 35.65; H, 3.60. $C_8H_7NaO_6S{\cdot}H_2O$ requires C, 35.30; H, 3.35%).

2-Hydroxy-1-naphthaldehyde-6-sulphonate (nals) was prepared from 2-hydroxy-1-naphthaldehyde (25 g, 0.145 mol) which was carefully dissolved in 98% H_2SO_4 (125 cm^3). The solution was stirred at 40 °C for 16 h, and then poured onto ice (400 cm^3). The mixture was warmed to 70 °C and then filtered. To the hot filtrate was added NaCl (80 g) and the solution cooled to 5 °C. The pink solid was filtered off and washed with an aqueous solution of NaCl (20%), followed by cold H_2O, and finally ethanol. The solid was recrystallized from H_2O

Table 1. Comparative data for the Fe^{III} complexes

Ligand[a]	pH	pFe^{3+}[b]	Approx. time to reach equilibrium[c] (weeks)	Low spin species (%)[d]	$E_{\frac{1}{2}}$[e]/V
L^1	6.66	22.8_5	10		
	7.50	25.5_4	7	77	-0.49_2
	8.27	27.5_3	3		
L^2	7.50	25.1_2	7	68	-0.51_3
L^3	7.50	24.9_9	2	75	-0.53_9
L^4	7.50	25.2_6	7	87	-0.47_0
L^5	7.50	⩾26.2	22	21	-0.38_4
L^6	7.50			82[f]	
L^7	7.50	⩾26.2	32		-0.73_8
L^8	7.50	26.1_1	8		
L^9	8.07	23.9_2			irrev.
Ferrichrome	7.50	25.49[g]			−0.687[h]
Ferrioxamine B	7.50	26.78[g]			−0.695[h]
edta	7.50	23.58[g]			−0.124[i]
dtpa	7.50	24.14[g]			
Transferrin	7.50	23.6[j]			−0.64[k]

[a] See Schemes 1 and 2 for definition of ligands; L^6 is the condensation product of nals and trien. [b] Conditions: $[L]_t = 5 \times 10^{-3}$, $[Fe^{III}]_t = 5 \times 10^{-4}$ mol dm^{-3}; 25 °C. [c] For the spectrophotometric competition reactions with dtpa or edta to go to completion. [d] At 25 °C. [e] At 20 ± 2 °C; values *versus* s.c.e. [f] At 35 °C. [g] Calculated from data given in ref. 17. [h] S. R. Cooper, J. V. McArdel, and K. N. Raymond, *Proc. Natl. Acad. Sci. USA*, 1978, **75**, 3551. [i] G. Schwarzenbach and J. Heller, *Helv. Chim. Acta*, 1951, **34**, 576. [j] R. Aasa, B. G. Malstrom, P. V. Sultman, and T. Uanagard, *Biochim. Biophys. Acta*, 1963, **75**, 203. [k] D. C. Harris, A. L. Rinehart, D. Hereld, R. W. Schwartz, P. Frances, and A. P. Salvador, *Biochim. Biophys. Acta*, 1985, **838**, 295.



	X	Y	Z	R	n
$[ML^7]^{3-}$	SO_3^-	H	H	CMe	1
$[ML^8]^{3-}$	SO_3^-	H	OMe	CMe	1
$[ML^9]^{3-}$	SO_3^-	H	H	N	2

Scheme 2. $M^{3+} = Fe^{3+}$

(activated charcoal) and then dried *in vacuo* at 90 °C, yielding the product (20.5 g, 52%) (Found: C, 47.55; H, 2.65. $C_{11}H_7NaO_5S$ requires C, 48.20; H, 2.55%). ^1H N.m.r. (D_2O): δ 10.07 (1 H, s, CHO), 7.96 (1 H, d, H^8), 7.89 (1 H, d, H^5), 7.67 (1 H, dd, H^7), and 6.71 (1 H, d, H^3).

The ligands tame,[11] dmtrien,[12] and sala[13] were prepared by literature methods and gave satisfactory analyses.

Physical Measurements.—The magnetic moments, μ, of Fe^{III} in the complexes were measured by an n.m.r. method[14,15] at 270 MHz on a JEOL GSX270 spectrometer. A Wilmad 517 coaxial tube was used, and the solvent was t-butyl alcohol (1%) in a mixture of D_2O (lock signal) and H_2O (30:70 v/v). The Fe^{III} solution (with a 50% excess of ligand) was contained in one compartment of the n.m.r. tube and an equal concentration of the ligand at the same pH in the other.

For a superconducting solenoid the shape factor, as previously defined,[14] is $-4\pi/3$ (ref. 16) rather than the value of $2\pi/3$ appropriate for a conventional magnet. The expression previously given[15] for the magnetic moment (μ) of a paramagnetic metal in a complex should therefore be modified to that given below, where f is the oscillator frequency (MHz) of

$$\mu = 0.0618(\Delta f \cdot T/2f \cdot M)^{\frac{1}{2}}$$

the superconducting spectrometer, T is the absolute temperature, M is the molar concentration of the metal ion, and Δf is the difference in frequency (Hz) between the two reference signals, with the signal from the paramagnetic solution being higher in frequency.

Cyclic voltammetry measurements were made using an Oxford Electrodes wave generator and potentiostat. U.v.–visible spectra were recorded on Perkin-Elmer 402, Perkin-Elmer 551, and Shimadzu UV-160 spectrometers. For the variable-temperature measurements a thermostatted cell holder was used. Proton n.m.r. spectra were recorded on Bruker WM250FT (250 MHz) and JEOL GSX270 (270 MHz) spectrometers.

Spectrophotometric competition reactions between $[Fe^{III}(dtpa)]^{2-}$ or $[Fe^{III}(edta)]^-$ and the various Schiff-base ligands (L) were performed with $[Fe^{III}]_t = 0.0005$ mol dm^{-3}, $[L]_t = 0.005$ mol dm^{-3} (t = total), and the concentration of dtpa or edta in the range 0.001—0.1 mol dm^{-3}. Absorbance measurements were taken at the appropriate $\lambda_{max.}$ for the Fe^{III}–Schiff

base species (in the range 470—550 nm), or at the isosbestic point for the spin-equilibrium systems, after the samples had been allowed to come to equilibrium, in the dark, at 25 °C. The slowest system to equilibrate involved $[Fe^{III}(dtpa)]^{2-}$ and L^7 and required 8 months while the fastest, involving L^3 and $[Fe^{III}(dtpa)]^{2-}$, required 2 weeks (Table 1). The solutions were buffered using 0.1 mol dm^{-3} biological buffers, 3-(*N*-morpholino)propanesulphonic acid (mops) or *N*-2-hydroxyethylpiperazine-*N'*-3-propanesulphonic acid (hepps), adjusted to the desired pH with KOH. The protonation and Fe^{III} ion formation constants for dtpa and edta were taken from Anderegg *et al.*[17]

Competition reactions between $[Ga^{III}(dtpa)]^{2-}$ and L^1 or L^7 where $[Ga^{III}]_t = 0.005$, $[dtpa]_t = 0.5$, $[L]_t = 0.006$ mol dm^{-3}, at pH 7.5, were followed by ^1H n.m.r. spectroscopy, at 25 °C. The aqueous solution of known pH was evaporated to dryness *in vacuo*, the residue dissolved in an equal volume of D_2O, and left until equilibrium had been established. The pH values quoted refer to those of the original aqueous solutions. The equilibrium was reached after 12 weeks for L^7 and 5 weeks for L^1. The intensities of the imine band for the complex relative to the imine and aldehyde bands for the free ligand species were used to calculate the concentrations of the Ga^{III} Schiff-base complexes. The protonation and Ga^{III} ion formation constants for dtpa were taken from the literature.[17,18]

The degree of hydrolysis of the Schiff base L^5, in D_2O at pH 7.94 and 25 °C, was determined using ^1H n.m.r. By varying the concentration of sals, in the range 0.1—0.4 mol dm^{-3}, and the concentration of dmtrien, in the range 0.1—0.2 mol dm^{-3}, it was possible to identify the imine or aldehyde band for each species and thus, from the ratio of the integrals of these bands, calculate the concentration of all the species present. A similar procedure was employed for the L^9 system at pH 7.90 and 25 °C.

Ligand Exchange Kinetics.—The kinetics were followed spectrophotometrically by measuring the increase in absorbance due to the formation of the Fe^{III} Schiff-base complexes, at $\lambda = 562$ nm (isosbestic point) for L^1 and $\lambda = 500$ nm for L^5, where the $[Fe^{III}(edta)]^-$ absorption is negligible.

Solutions of $[Fe^{III}(edta)]^-$ were prepared by mixing a stock solution of $Fe(NO_3)_3 \cdot 9H_2O$ with edta (10% molar excess). Use of a 10% excess of edta did not affect the exchange reaction rates. For reactions with L^1 $[Fe^{III}(edta)^-] = 4 \times 10^{-4}$, [sals] = 8×10^{-3} to 4.8×10^{-2}, [trien] = 4×10^{-3} to 2.8×10^{-2} mol dm^{-3}; with L^5 $[Fe^{III}(edta)^-] = 3 \times 10^{-4}$, [sals] = 6×10^{-3} to 2.4×10^{-2}, [dmtrien] = 3×10^{-3} to 2.4×10^{-2} mol dm^{-3}. A constant pH was maintained using 0.1 mol dm^{-3} hepps buffer, the pH being measured before and directly after each run. All runs were performed at 25 °C.

Results and Discussion

The Ligands.—Formation of the Schiff bases, from the constituent aldehyde (S) and amine (A), is not complete in aqueous solution. The Schiff bases derived from trien or dmtrien (L^1—L^5) exist in the equilibria (1),* in aqueous solution.

$$A + 2S \underset{}{\overset{K_1}{\rightleftharpoons}} AS + S \overset{K_2}{\rightleftharpoons} AS_2 = [H_2L]^{2-} \quad (1)$$
$$K_1 = [AS]/[A][S], \quad K_2 = [AS_2]/[AS][S]$$

From ^1H n.m.r. spectra of a mixture of sals and dmtrien (the L^5 system) in D_2O, the values of K_1 and K_2 were 166 ± 8 dm^3 mol^{-1} and 31 ± 1.5 dm^3 mol^{-1} respectively at pH 7.94 and 25 °C. However the ^1H n.m.r. spectra of the L^1 system, in D_2O, are far more complex and it was not possible to determine

* The species AS, AS_2, and AS_3 represent only the stoicheiometries of the species obtained by combination of A and S; no account is taken of loss of H_2O in their formation and charges are ignored.

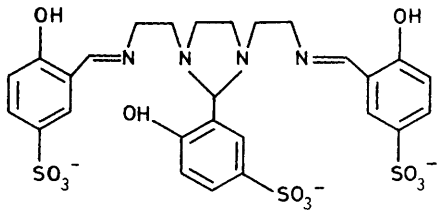


Figure 1. Sals:trien 3:1 species

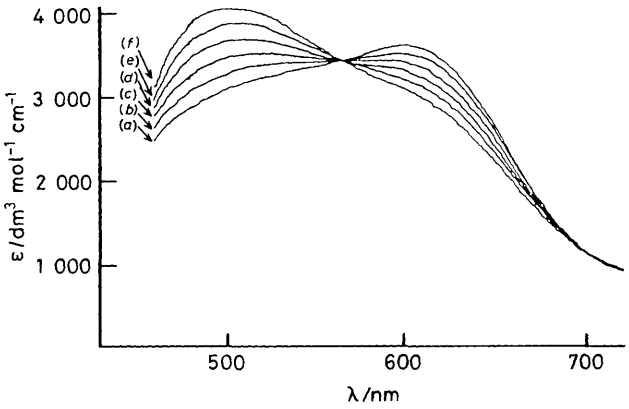


Figure 2. Variable temperature u.v.–visible spectrum of $[Fe^{III}L^1]^-$ in aqueous solution, at pH 8.0: (*a*) 15, (*b*) 25, (*c*) 35, (*d*) 45, (*e*) 55, (*f*) 65 °C

the equilibrium constants. The complexity of the spectra can be attributed to the presence of the two secondary amine nitrogens in the trien, which can lead to the formation of a 3:1 sals:trien species in which the aromatic rings are non-equivalent (see Figure 1). A related species has been isolated from salicylaldehyde and trien[19] and a binuclear Fe^{III} complex containing that ligand has been reported.[20]

For the Schiff bases derived from tame or tren (L^7 – L^9) the equilibria (2) exist in aqueous solution. From the ^1H n.m.r.

$$A + 3S \underset{}{\overset{K_1}{\rightleftharpoons}} AS + 2S \overset{K_2}{\rightleftharpoons} AS_2 + S \overset{K_3}{\rightleftharpoons} AS_3 = [H_3L]^{3-} \quad (2)$$
$$K_1 = [AS]/[A][S], \quad K_2 = [AS_2]/[AS][S], \quad K_3 = [AS_3]/[AS_2][S]$$

spectra of the L^9 system, in D_2O, the values of K_1, K_2, and K_3 were 231 ± 11 dm^3 mol^{-1}, 162 ± 8 dm^3 mol^{-1}, and 32 ± 1.5 dm^3 mol^{-1} respectively, at pH 7.90 and 25 °C. The ^1H n.m.r. spectra of the L^7 system, in D_2O, had all the imine bands coincident preventing determination of the equilibrium constants.

The Iron(III) Complexes.—Formation of the Fe^{III} complexes was followed by u.v.–visible spectroscopy. The complexes were formed at pH $\geqslant$ 4 and were stable up to pH 10 for all the ligands except L^9, for which the complex began to breakdown at pH > 8.5. The constancy of the spectra over this wide pH range suggests that water is not co-ordinated to the Fe^{III} ion, in contrast to the $[Fe^{III}(edta)(H_2O)]^-$ complex which loses a proton with $pK_a = 7.50$.[17]

The u.v.–visible spectra of the complexes derived from trien or dmtrien, *i.e.* with an N_4O_2 donor set, proved to be temperature dependent, containing two maxima and an isosbestic point. Figure 2 shows the spectrum of $[FeL^1]^-$ as a function of temperature. Magnetic susceptibility measurements confirm that a high-spin/low-spin equilibrium is involved with these complexes at 25 °C. Taking values of $\mu_B = 1.94$ and 5.92

for low-spin and high-spin Fe^{III} respectively[21] the proportion of low-spin Fe^{III} present in the complexes can be calculated (see Table 1). The variation of the proportion of low-spin Fe^{III} within the series of N_4O_2 complexes arising from the different aldehyde substituents is in agreement with the work of Tweedle and Wilson[21] on the analogous non-water-soluble Fe^{III} complexes derived from neutral salicylaldehydes and trien. They proposed that the aldehyde substituent effect must be electronic in origin since there is no obvious intramolecular steric interaction (see Figure 1). The variations are therefore explained by the assumption that π-acceptance by the ligands is more important than the σ-donor capabilities. Thus electron-withdrawing substituents will increase the proportion of low-spin Fe^{III}. In contrast the much lower proportion of low-spin Fe^{III} in $[FeL^5]^-$ is probably due to steric interactions from the methyl groups, which will reduce the ligand field.

The results reported here are also consistent with the solvent dependency of the related organic-soluble systems in non-aqueous solvents,[21] where polar solvents favour the low-spin species.

The u.v.–visible spectra of the complexes derived from tame or tren, *i.e.* with an N_3O_3 donor set, exhibit only one phenol–Fe^{III} charge-transfer band, in the visible region, and the magnetic susceptibility measurements show that the complexes are entirely high-spin.

The colours of the Fe^{III} complexes in aqueous solution, at room temperature, give some indication of the position of the spin equilibrium. The predominantly low-spin L^6 complex is green, the higher spin L^1 complex is purple grey, and the even higher spin L^5 complex is red, as is the entirely high-spin L^7 complex.

Stability of the Complexes.—Since the Schiff-base ligands exist in a formation/hydrolysis equilibrium in aqueous solution it would be very difficult to measure the stability constants of their Fe^{III} complexes by the normal method of potentiometric titrations. However pFe^{3+} values (where $pFe^{3+} = -\log_{10}[Fe^{3+}]_{free}$) were readily determined by competition equilibria with edta and dtpa (see Table 1). The pFe^{3+} values are of more practical significance than stability constants since they express the effective binding strength of a ligand at a particular pH. These values therefore allow the direct comparison of the efficiency of different ligands in sequestrating the Fe^{III} ion under the same conditions.

From Table 1 one can see that the pFe^{3+} values for the complexes are in the order: $[Fe^{III}L^7]^{3-} \approx [Fe^{III}L^5]^- > [Fe^{III}L^1]^- \gg [Fe^{III}L^9]^{3-}$. The complexes, with the exception of $[Fe^{III}L^9]^{3-}$, have pFe^{3+} values of the same order as those of the hydroxamic siderophores ferrichrome and ferrioxamine B, and much greater than those of edta, dtpa, and the iron transport protein transferrin. The much lower pFe^{3+} value for the $[Fe^{III}L^9]^{3-}$ complex is consistent with the earlier observation of the breakdown of the complex at pH > 8.5. This may be due to the greater flexibility in the longer amine backbone leading to a greater loss of entropy in forming the complex.

It is noticeable that within the series of ligands with an N_4O_2 donor set there is no correlation between the pFe^{3+} value of the complex and the proportion of low-spin Fe^{III} present. It therefore appears that there is no simple relationship between the ligand field produced by the ligand and the stability of the complex formed.

Electrochemistry.—Cyclic voltammograms of all the Fe^{III} complexes, except $[FeL^9]^{3-}$, exhibit reversible waves with potentials which are pH independent over the range 5—10 (see Table 1). The observed peak separation of 60 mV and the peak cathodic to aniodic currents of nearly unity are consistent with one-electron electrochemical reversibility.

The reduction potentials for all the N_4O_2 donor complexes are similar and in the range −0.38 to −0.54 V *vs.* saturated calomel electrode (s.c.e.). These values are more negative than the reduction potential for $[Fe^{III}(edta)]^-$ but less negative than the potentials for the ferrioxamine B and ferrichrome complexes. For the related Fe^{III} Schiff-base complexes derived from neutral salicylaldehydes it has been shown that the reduction produced high-spin Fe^{II} complexes, regardless of the position of the Fe^{III} spin equilibrium.[22]

The reduction potential of -0.73_8 V (*vs.* s.c.e.) for $[FeL^7]^{3-}$ is the most negative of those reported here and is more negative than those for the hydroxamic acid siderophore complexes. The ligand L^7, with its N_3O_3 donor set, greatly favours the Fe^{III} complex relative to that of Fe^{II}.

Reversible reduction waves were not observed for the Fe^{III} complex of L^9, the other N_3O_3 donor complex, presumably due to the breakdown of the Fe^{II} complex produced. This instability of the Fe^{II} complex of L^9 is consistent with the much lower stability of the Fe^{III} complex, compared to the other Fe^{III} Schiff-base complexes, as noted earlier.

Kinetics of Ligand Replacement.—The mechanism by which one multidentate ligand displaces another from a metal ion [*e.g.* equation (3)] is thought to be dependent upon the ability of both ligands to co-ordinate the metal ion simultaneously.[23] Once the incoming ligand has gained a co-ordination foothold the ligand exchange proceeds by successive unwrapping of the outgoing ligand, with increasing co-ordination of the incoming ligand. Due to the formation/hydrolysis equilibrium existing

$$[Fe^{III}(edta)]^- + [H_2L^5]^{2-} \rightleftharpoons [Fe^{III}L^5]^- + H_2edta^{2-} \quad (3)$$

for the L^5 system in aqueous solution there are four initial rate-determining steps by which the ligand exchange can proceed: equations (4)—(7). The pseudo-first-order rate constant for

$$[Fe^{III}(edta)]^- + S \longrightarrow \text{products} \quad (4)$$

$$[Fe^{III}(edta)]^- + A \longrightarrow \text{products} \quad (5)$$

$$[Fe^{III}(edta)]^- + AS \longrightarrow \text{products} \quad (6)$$

$$[Fe^{III}(edta)]^- + AS_2 \longrightarrow \text{products} \quad (7)$$

the disappearence of $[Fe^{III}(edta)]^-$ ($k_{obs.}$) is given by equation (8).

$$k_{obs.} = k_1[S] + k_2[A] + k_3[AS] + k_4[AS_2] \quad (8)$$

The reaction runs were all performed with at least a ten-fold excess of the Schiff base. Pseudo-first-order rate plots were obtained showing the reaction to be first order in $[Fe^{III}(edta)]^-$. At constant pH, $k_{obs.}$ is dependent upon the total sals ($[S]_t$) and dmtrien ($[A]_t$) concentrations (see Table 2). Assuming that the equilibrium constants for the L^5 system in D_2O will not differ appreciably from those in H_2O, one can quantitatively analyse the dependency of $k_{obs.}$ upon the concentrations of the various species present in the system.

A fit of the observed data to equation (8) yielded positive values for k_3 and k_4, with k_1 and k_2 being negative but of negligible absolute magnitude. This suggests that initial attack of $[Fe^{III}(edta)]^-$ by free sals or free amine contributes little, if at all, to ligand exchange. Application of a least-squares method to the data, assuming the reaction proceeds only through attack by AS or AS_2, yielded $k_3 = (2.0 \pm 0.3) \times 10^{-3}$ dm^3 mol^{-1} s^{-1} and $k_4 = (5.7 \pm 0.9) \times 10^{-3}$ dm^3 mol^{-1} s^{-1} (the quoted errors are based upon the effect of variations in these parameters on the deviations between $k_{obs.}$ and $k_{calc.}$). These values were used to

Table 2. The observed ($k_{obs.}$) and calculated ($k_{calc.}$) pseudo-first-order rate constants at various sals and dmtrien concentrations, at pH 7.94, for reaction (3)

$10^3[S]_t$/ mol dm^{-3}	$10^3[A]_t$/ mol dm^{-3}	$10^3[S]_{calc.}$/ mol dm^{-3}	$10^3[A]_{calc.}$/ mol dm^{-3}	$10^3[AS]_{calc.}$/ mol dm^{-3}	$10^3[AS_2]_{calc.}$/ mol dm^{-3}	$10^6k_{obs.}$/ s^{-1}	$10^6k_{calc.}$/ s^{-1}
6.0	3.0	4.46	1.63	1.21	0.16_8	4.7	3.4
12.0	3.0	9.52	0.98_3	1.55	0.46_3	6.2	5.7
24.0	3.0	20.5	0.45_6	1.55	0.99_3	8.2	8.7
6.0	6.0	3.45	3.67	2.10	0.22_7	6.2	5.5
6.0	12.0	2.29	8.52	3.24	0.23_3	8.2	7.8
6.0	24.0	1.33	19.5	4.31	0.18_0	8.7	9.6

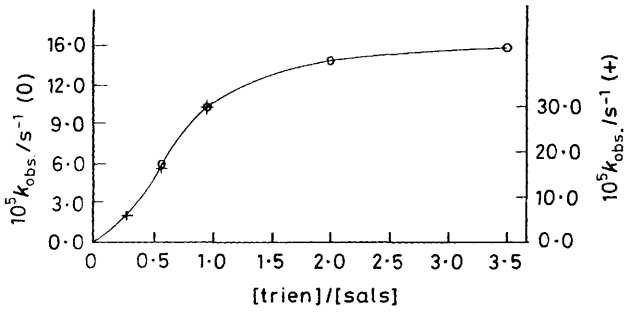


Figure 3. A plot of the pseudo-first-order rate constant ($k_{obs.}$) *vs.* [trien]/[sals], at constant pH and [sals], for reaction (9): pH 7.90; [sals] = 0.008 (O), 0.016 mol dm^{-3} (+)

Table 3. Pseudo-first-order rate constants ($k_{obs.}$) at various sals:amine concentration ratios, at pH 7.90, for reaction (9)

$[trien]_t$/mol dm^{-3}	$\frac{[sals]_t}{[trien]_t}$	$10^5k_{obs.}$/s^{-1}
0.016	0.5	16.1
	1.0	28.4
	2.0	32.3
0.008	1.0	11.9
	2.0	15.9
	4.0	10.1
0.004	2.0	7.5
	4.0	6.2
	8.0	4.3
	16.0	4.0

calculate the observed rate constants and a reasonable fit was obtained (see Table 2). A reasonable fit cannot be achieved if one assumes that there is only one pathway leading to ligand exchange.

The unreactive nature of free dmtrien (A) and free sals (S) could be due to the lifetimes of any Fe^{III}(edta)A and Fe^{III}(edta)S species being insufficient to allow the Schiff-base formation required to complete ligand exchange. The appearance of separate imine bands for the different Schiff-base species in the ^1H n.m.r. spectra of the L^5 system shows Schiff-base formation and hydrolysis to be slow on the n.m.r. time-scale. The Schiff-base species AS and AS_2 should produce complexes with longer lifetimes since they are of high denticity and contain the phenoxy group which will bind strongly to the Fe^{III}. The higher reactivity of AS_2, compared to AS, suggests that the phenoxy group may be involved in the initial attack.

$$[Fe^{III}(edta)]^- + [H_2L^1]^{2-} \rightleftharpoons [Fe^{III}L^1]^- + H_2edta^{2-} \quad (9)$$

The kinetics for the L^1 system, equation (9), also proved to be pseudo-first-order in $[Fe^{III}(edta)^-]$ and dependent upon the total sals and trien concentrations at constant pH. However, as a result of the formation of a 3:1 sals:trien species (AS_3), equilibrium constants for the L^1 system have not been determined (see earlier). Thus the results can only be analysed in a qualitative manner. This is most effectively performed by observing the dependence of $k_{obs.}$ upon the trien:sals concentration ratio ([A]:[S]).

As the [A]:[S] ratio increases, at constant sals concentration, $k_{obs.}$ initially increases rapidly but then levels off to a maximum at high [A]:[S] ratios (see Figure 3). These results suggest that the free amine is unreactive since at high [A]:[S] ratios the rate constant does not increase with increasing amine concentration. The results also suggest that the 1:1 sals:amine species (AS) is involved in a reactive pathway, since at high [A]:[S] ratios AS will be the major species present, other than free amine.

As the [S]:[A] ratio increases, at constant trien concentration, $k_{obs.}$ initially increases to a maximum (at [S]:[A] ≈ 3) but then falls off, tending to a non-zero value at high [S]:[A] ratios (see Table 3). This suggests that the AS_3 species is involved in a reactive pathway, since at high [S]:[A] ratios it will begin to dominate, but that it reacts at a lower rate than AS or AS_2 (which will dominate at lower [S]:[A] ratios). The reduced reactivity of AS_3 could be due to steric blocking of the two central secondary nitrogens by the third sals group attached. The reactivity might also be reduced by the need for the hydrolytic loss of a sals group from the ligand, before ligand exchange can be completed.

These qualitative results are consistent with the quantitative results obtained for the L^5 system, but with the added complexity due to the presence of the 3:1 sals:amine species. In spite of this complexity it is clear that ligand exchange occurs much more slowly with L^5 than with the corresponding L^1 system (see Tables 2 and 3). This may be due to steric interference by the *N*-methyl groups in the reactive AS and AS_2 species of the L^5 system.

The values of $k_{obs.}$, at constant sals and trien concentrations, increase greatly as the pH increases, in the range pH 7—8.5. The magnitude of this pH dependence is too great to be solely due to a shift in the La^1 hydrolysis/formation equilibrium position with increase in pH. There must therefore be a deprotonation of one, or more, of the reactants resulting in increased reactivity.

Assuming that only one deprotonation is important, we can write equations (10)—(12). If $k \gg k'$, then at pH near pK_a, $k_{obs.}$ is given by equation (13) where *C* is a constant.

$$HA \overset{K_a}{\rightleftharpoons} H^+ + A^- \quad (10)$$

$$A^- \xrightarrow{k} \text{products} \quad (11)$$

$$HA \xrightarrow{k'} \text{products} \quad (12)$$

$$(k_{obs.})^{-1} = [H^+]/CK_a + 1/C \quad (13)$$

A plot of the inverse of $k_{obs.}$ *versus* the hydrogen ion concentration gives a reasonably good straight line, with a value of $pK_a = 7.9$ (see Figure 4). The pK_a values for both $[Fe^{III}(edta)(H_2O)]^-$ ($pK_a = 7.50$)[17] and those expected for the Schiff-base species [based upon pK_a(sals) = 7.37][24] are close to this value. It has been suggested that deprotonation of $[Fe^{III}(edta)(H_2O)]^-$ activates the complex towards ligand exchange with ferrioxamine B.[25] However, due to the complexity of this system, with the additional possibility of a shift in the Schiff-base formation equilibria, it is not possible to attribute the pH dependence to any one process.

Gallium(III) *Complexes.*—The formation of Ga^{III} complexes of L^1, L^5, and L^7, in D_2O, was studied by ^1H n.m.r. at pH 7.5. The alkyl region (δ 2—4.5) of the $[GaL^1]^-$ ^1H n.m.r. spectrum has five multiplets, four of intensity 2 H and one of intensity 4 H (see Figure 5). This is consistent with the rigid structure shown in Scheme 1 in which there are six different pairs of methylene protons, with two pairs producing coincident bands in the ^1H n.m.r. spectrum. The ^1H n.m.r. spectrum of $[GaL^5]^-$ is similar but with an extra singlet resonance, at δ 2.22 (intensity 6 H), from the additional methyl groups present.

The alkyl region of the $[GaL^7]^{3-}$ ^1H n.m.r. spectrum shows an AB pair of doublets, δ_A 4.18 (3 H) and δ_B 3.65 (3 H), and a singlet, δ 1.17 (3 H) (see Figure 6). This is consistent with the rigid structure shown in Scheme 2, in which the CH_2 groups are asymmetric.

These structures have also been found by *X*-ray diffraction for the related Ni^{II} and Ga^{III} complexes of the unsulphonated analogues of L^1 (ref. 26) and L^8 (ref. 4), respectively.[26]

The magnitudes of pGa^{3+} for $[GaL^1]^-$ and $[GaL^7]^{3-}$ were found to be 24.5_4 and 24.6_4 respectively at pH 7.50, 25 °C, $[Ga^{III}]_t = 0.004$ and $[L]_t = 0.004$ mol dm^{-3}. These values are much greater than those reported for the Ga^{III} complexes of dtpa, $pGa^{3+} = 22.5_8$ (same conditions)[18] and transferrin, $pGa^{3+} = 21.3$ (pH 7.4, 5×10^{-3} mol dm^{-3} bicarbonate),[27] although any comparison can be only approximate due to the isotope effect on going from D_2O to H_2O.

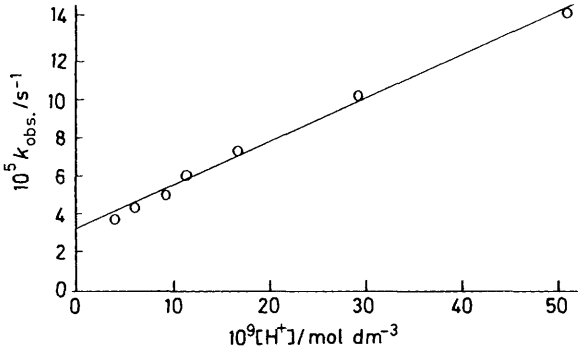


Figure 4. A plot of the inverse of the pseudo-first-order rate constant *versus* hydrogen ion concentration for reaction (9)

Conclusions

The Schiff-base ligands L^1, L^5, and L^7 form very stable, highly water-soluble, Fe^{III} and Ga^{III} complexes at physiological pH. However these ligands are slow to displace the multidentate ligands edta and dtpa from the metal ions. This suggests that the ligands would be of little use in iron overload treatment, as they would probably also be slow in removing the Fe^{III} ion from the transport protein transferrin. However since the ligands form complexes which are not only thermodynamically stable, but also kinetically inert over short periods, their Ga^{III} complexes, or those of related Schiff bases, may be of use in certain biomedical applications where both of these properties are

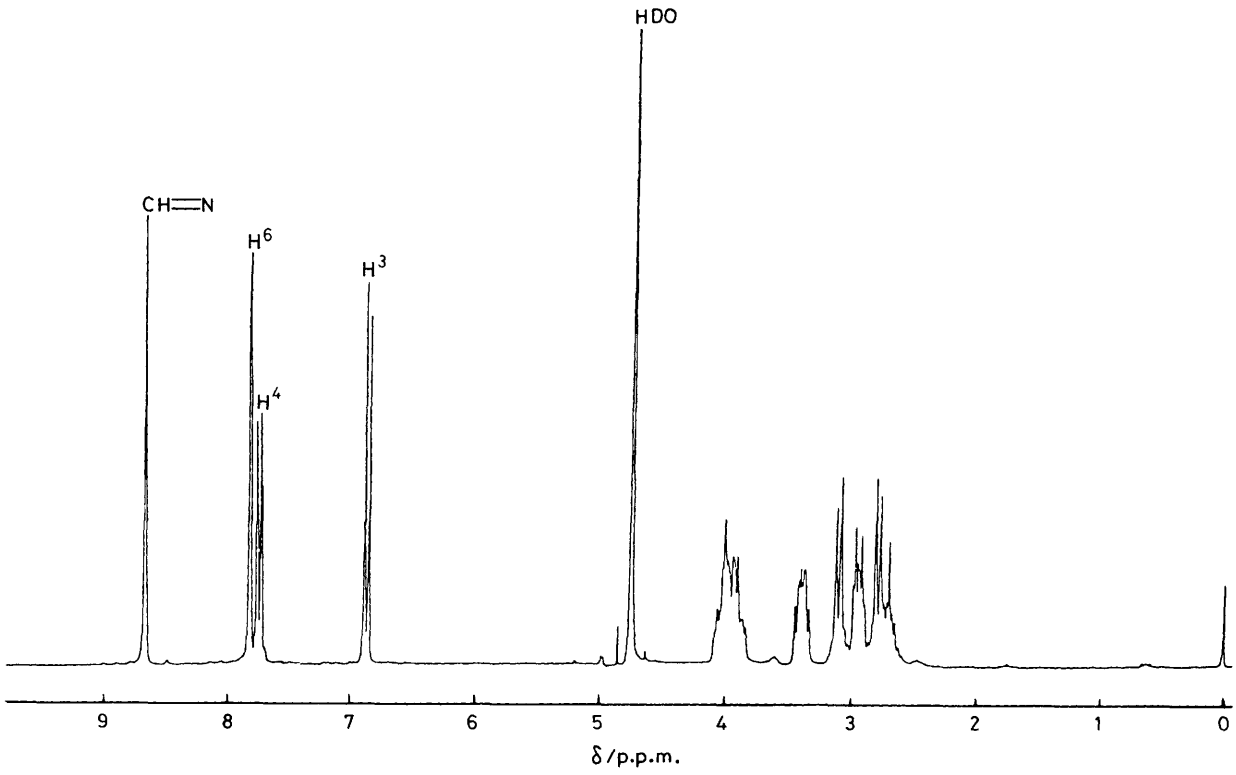


Figure 5. Proton n.m.r. spectrum of $[Ga^{III}L^1]^-$ in D_2O, at pH 7.5 (270 MHz)

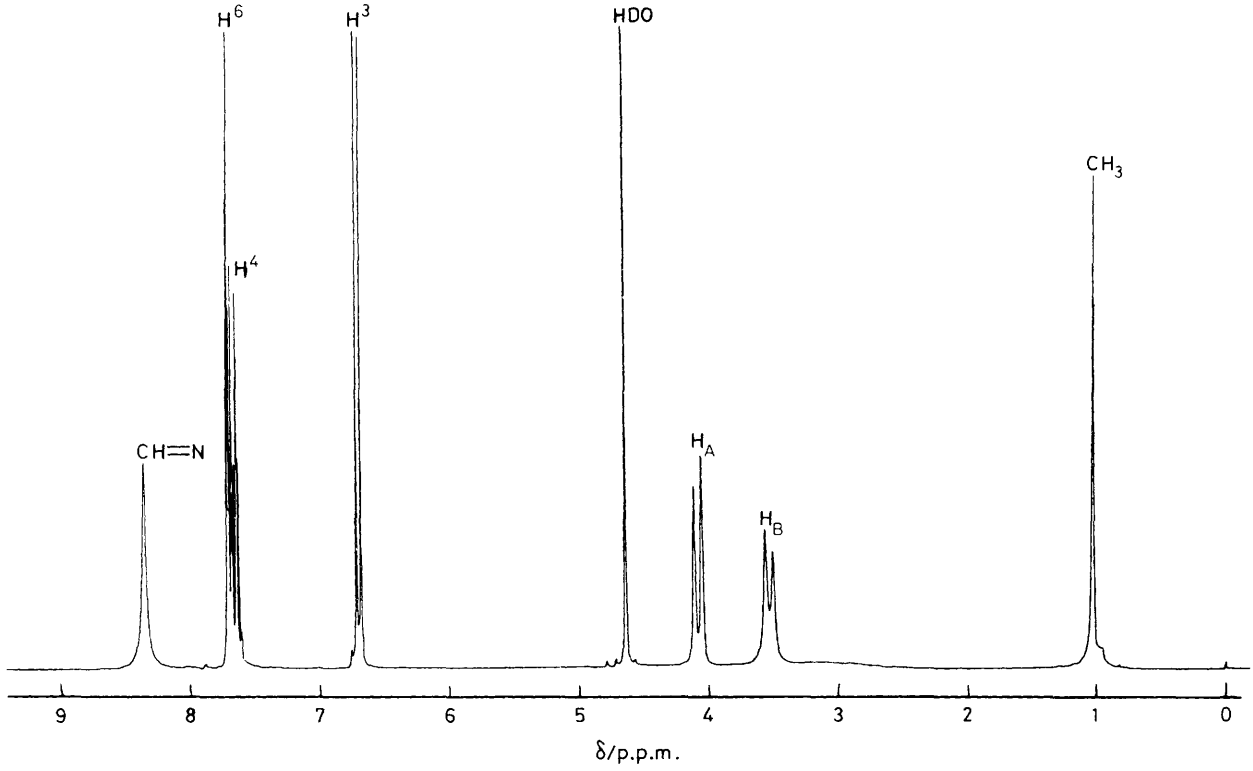


Figure 6. Proton n.m.r. spectrum of $[Ga^{III}L^{7}]^{3-}$ in D_2O, at pH 7.5 (270 MHz)

desirable. The versatility of the ligands (modifications to the complete ligands can easily be effected by simple alterations to the constituent aldehyde and/or amine) is another desirable property.

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