34. Metal Complexes with Macrocyclic Ligands

Part XXXVI[1])

Thermodynamic and Kinetic Studies of Bivalent and Trivalent Metal Ions with 1,4,7,10-Tetraazacyclododecane-1,4,7-triacetic Acid

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NMR, potentiometric, and UV/VIS measurements were run to study the protonation and the In^{3+} and Cu^{2+} stability constants of 1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid (do3a, L). The protonation of do3a follows the typical scheme with two high and several low log K_H values. Between pH 11 and 13, the protonation mainly occurs at the N-atom, which is not substituted by an acetate side chain. The In^{3+} complex is not appreciably protonated even at low pH values (pH $\sim$1.7), whereas [CuL] can add up to three protons in acidic solution to give the species [CuLH], [$CuLH_2$], and [$CuLH_3$], the stability of which was determined. The formation rates of the Y^{3+}, Gd^{3+}, Ga^{3+}, and In^{3+} complexes with do3a were measured using a pH-stat technique, whereas that of Cu^{2+}, being faster, was followed on a stopped-flow spectrophotometer. In all cases, the reaction scheme implies the rapid formation of partially protonated intermediates, which rearrange themselves to the final product in the rate-determining process. $([MLH])_{in}$, an intermediate, in which the metal ion probably is coordinated by two amino acetate groups, proved to be the reactive species for Y^{3+}, Gd^{3+}, and Ga^{3+}. The formation of [Cu(do3a)] was interpreted by postulating that either $([CuLH])_{in}$ or $([CuLH])_{in}$, and $([CuLH_2])_{in}$ are the reactive complexes. The rates of dissociation of the Y^{3+}, Gd^{3+}, and Cu^{2+} complexes with do3a were studied spectrophotometrically. For Y^{3+} and Gd^{3+}, arsenazo III was used as a scavenger, whereas for Cu^{2+} the absorption associated with d-d* transition was followed. For [Y(do3a)] and [Gd(do3a)], the rate law follows the kinetic expression $k_{obsd} = k_0 + k_1[H^+]$. The dissociation of [Cu(do3a)] goes through the proton-independent dissociation of [$CuLH_3$], which is the main species at low pH.

Introduction. – The field of macrocyclic complexes has gone through a rapid expansion in different directions. One of these has been the functionalization, achieved by the introduction of side chains carrying a donor group, which can act as an additional ligand for the metal ion. These compounds show interesting properties with respect to their complexation ability, selectivity, coordination geometry, and have potential applications [2]. Some of them are among the strongest chelators known, thus making them useful for medical applications, for which kinetical inertness and thermodynamical stability are of paramount importance. So, applications of macrocyclic metal complexes as contrast agents in magnetic resonance imaging (MRI) [3] and for labelling monoclonal antibodies have been described [4].

The Gd^{3+} complexes of dota (= 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) and of dtpa (diethylenetriaminepentaacetic acid) are two of the MRI contrast agents already in use [5]. To obtain new, stable, and nonionic Gd^{3+} chelates, *Tweedle* and

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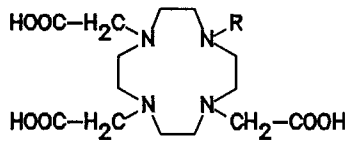
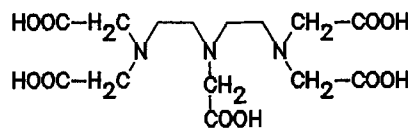


do3a R = H
dota R = CH_2COOH



dtpa

coworkers [6] [7] have prepared 1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid (do3a), which contains a secondary N-atom accessible for further derivatization. Compared to $[Gd(dtpa)]^{2-}$ and $[Gd(dota)]^{-}$, the Gd^{3+} complex of do3a has similar chemical and biological properties such as high thermodynamic stability, slow dissociation kinetics, high relaxation effectiveness, but a lower osmolality, because, in contrast to the other complexes, it is not charged. In addition, dtpa-, dota-, and do3a-modified antibodies, which were labelled with Y^{3+} and In^{3+}, have been described in the literature and indicate a potential application for tumor diagnosis and therapy [4]. Since the basic requirement for complexes to be used in medicine is that they must be thermodynamically stable and kinetically inert, it was of interest to perform a basic study of the kinetic properties of the do3a complexes with metal ions such as Y^{3+}, Ga^{3+}, In^{3+}, Gd^{3+}, and Cu^{2+}, all of which have potential applications as radionuclides (Y^{3+}, Ga^{3+}, In^{3+}, and Cu^{2+}) or as MRI contrast agents (Gd^{3+}).

Experimental. – *Reagents.* 1,4,7,10-Tetraazacyclododecane-1,4,7-triacetic acid (do3a) was synthesized according to [7]. The monohydrochloride was recrystallized from H_2O/acetone, but it contained (^{1}H-NMR), a small amount of acetone, which could not be removed even *in vacuo*. Anal. calc. for $C_{14}H_{26}N_4O_6 \cdot 1.08\ HCl \cdot 0.90H_2O \cdot 0.29C_3H_6O$ (mol. wt.: 418.8): C 42.55, H 7.54, Cl 9.12, N 13.36, H_2O 3.86; found: C 42.85, H 7.15, Cl 8.82, N 13.06, H_2O 3.47.

Deuterium chloride DCl (*ca.* 20% in D_2O, isotopic purity > 99.5 atom-% D) and deuterium oxide D_2O (isotopic purity > 99.8 atom-% D) were obtained from *Dr. Glaser AG*. CsOD was prepared from $CsOH \cdot H_2O$, which was dissolved in D_2O and evaporated. The stock solns. of Gd^{3+} were prepared by dissolving Gd_2O_3 (*Merck*) in an excess of HNO_3. All other reagents were of anal. grade (*Merck* or *Fluka*).

pH-Potentiometric and Spectrophotometric Titrations. All potentiometric titrations were carried out at 25 ± 0.1° and I = 0.5M (KNO_3) using an automatic microprocessor-controlled potentiometric titrator [8]. The pH electrode was calibrated with two buffer solns. of pH 4 and pH 7 and checked daily. The potentiometric sample solns. were titrated with 0.4M NaOH. Typical concentrations were $[do3a] = 2.75 \cdot 10^{-3}$ M for the ligand titration, and $[do3a] = 2.5 \cdot 10^{-3}$ M and $[Cu^{2+}] = 2.25 \cdot 10^{-3}$ M for the Cu^{2+}/do3a titrations. The titration curves were fitted using program TITFIT [9].

The spectrophotometric titrations were performed at 25 ± 0.1° without controlling the ionic strength. They were run on an automatic computer-controlled spectrophotometric titrator consisting of a UV/VIS spectrophotometer (*Pye Unicam PU 8800, Philips*), a pH-meter (*Metrohm* E 605), a Dosimat (*Metrohm* E 665), and a thermostated water bath (*Haake*) [10]. The computer (*AT-286*) runs the titration by adding small amounts of the titrant (HNO_3 = 0.5M), monitoring the pH and reading the absorbance at fixed wavelengths. The data were evaluated with the program SPECFIT [11]. For the titration of [Cu(do3a)] with HNO_3 the concentrations were: $[Cu^{2+}] = 1 \cdot 10^{-3}$ M and $[do3a] = 1.1 \cdot 10^{-3}$ M.

NMR Measurements. Both ^{1}H- and ^{13}C-NMR spectra were recorded at 25° and 300 MHz on a *Varian GEM 300 NMR* instrument. All sample solns. were made up in D_2O. For the log K_H determination of do3a, a D_2O soln. with [do3a] = 0.2M was adjusted to pH values between 11 and 13 with CsOD. The ^{1}H-NMR spectrum shows four signals (A, B, C, and D) with a relative intensity 1:2:4:4. The chemical shifts of the four signals increase by 0.1, 0.075, 0.086, and 0.2–0.3 ppm, respectively, as the pH is increased from 11 to 13. The concentrations of In^{3+} and

do3a were both 0.2M (1:1 mixture), or 0.1M and 0.2M (1:2 mixture), respectively. The pD, adjusted with DCl and CsOD, was measured with a pH-meter (*Metrohm E 654*) equipped with a combined glass electrode. The final pH was calculated using the equation pH = pD – 0.4 [12].

Formation Kinetics. All measurements were performed at 25 ± 0.1° and I = 0.5M (KNO_3). The formations of [Y(do3a)], [Gd(do3a)], [Ga(do3a)], and [In(do3a)] were followed on a pH-stat instrument consisting of a pH-meter (*Metrohm E 510*), an Impulsomat (*Metrohm E 473*) and a Dosimat (*Metrohm E 415*). Typical concentrations were [do3a] = $4.1 \cdot 10^{-4}$M, while the concentration of the metal ions varied between $4.1 \cdot 10^{-4}$M and $6.0 \cdot 10^{-3}$M. The titrant NaOH (0.05M) was prepared from standard *Titrisol* (*Merck*) solns. The change of the pH during the reaction was Δ(pH) ≤ 0.02.

The formation of [Cu(do3a)] was measured on a *Durrum D-150* stopped-flow spectrophotometer equipped with a digital transient recorder (*Datalab DL901*). The reaction was followed at 780 nm, where the absorbance of [Cu(do3a)] is close to its maximum. 2,6-Dimethylpyridine-3-sulfonic acid was used as buffer [13] with concentrations between 0.125M and 0.15M (Δ(pH) ≤ 0.05), depending upon the pH and the concentrations of do3a and Cu^{2+}. The concentration of do3a was $1 \cdot 10^{-3}$M, while the concentration of Cu^{2+} varied between $3 \cdot 10^{-3}$M and $2 \cdot 10^{-2}$M.

Dissociation Kinetics. All measurements were carried out at 25 ± 0.1° and I = 0.5M (KNO_3) for [Y(do3a)] and [Gd(do3a)] or I = 1.0M (KNO_3) for [Cu(do3a)]. The dissociation rates of [Y(do3a)] and [Gd(do3a)] were determined in the presence of arsenazo III by measuring the increase in absorbance at 650 nm of the arsenazo III/metal complex [14]. The concentrations of metal ion and the ligand were $2 \cdot 10^{-5}$M, while that of arsenazo III varied between $3.0 \cdot 10^{-5}$M and $1.1 \cdot 10^{-4}$M, depending upon the pH of the sample. The dissociation of [Cu(do3a)] was directly followed measuring the decrease in absorbance of the Cu^{2+} chromophore at 650 nm during the reaction. The concentrations of Cu^{2+} and ligand were $1.0 \cdot 10^{-3}$M. All the dissociation measurements were performed on a *Perkin-Elmer Lamda 2* UV/VIS spectrophotometer equipped with a hand-driven mixing device, which allows to mix the two reactants in less than 0.5 s. The concentration-time curves were recorded and calculated as first-order reactions.

Results and Discussion. – *Potentiometric Titrations and NMR Spectra of do3a.* The first protonation constant K_1 of do3a, being relatively high (*Table 1*), was determined using the ^{1}H-NMR method in the pH range from 11 to 13. ^{1}H-NMR Spectra of do3a show three *singlets* (*A*, *B*, and *C*) and one *multiplet* (*D*). From the pH dependence of the chemical shift of the *singlet A*, one can determine K_1 using *Eqn. 1* (*Fig. 1*).

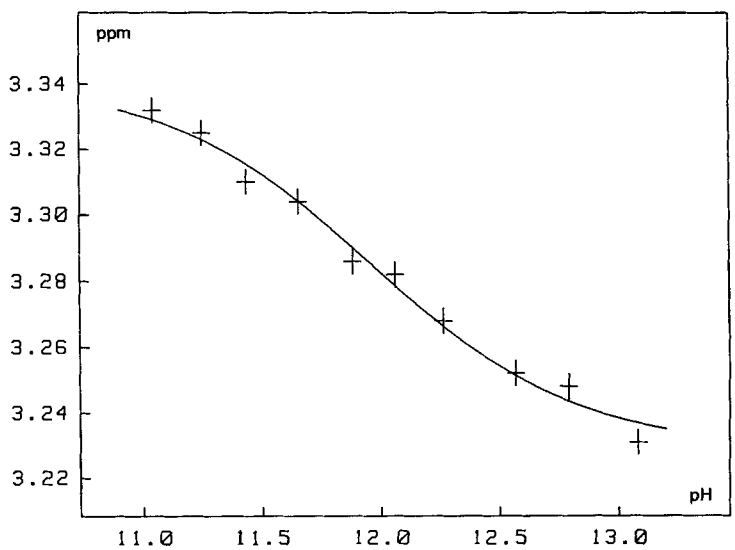
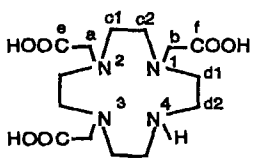


Fig. 1. *NMR Titration curve of 0.2M do3a: δ* [ppm] *vs. pH*

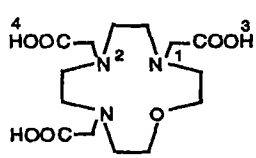
$$\delta = \frac{\delta_L[L] + \delta_{LH}[LH]}{C_L} = \frac{\delta_L + \delta_{LH}K_1[H^+]}{1 + K_1[H^+]} \quad (1)$$

where δ_L and δ_{LH} are the chemical shifts of L and LH, respectively. This expression is based on *Sudmeier* and *Reilley*'s proposal [15], that the total chemical shift equals the weighted mean value of the chemical shifts of the different species. The accuracy of the K_1 value determined by the ^{1}H-NMR method is, as mentioned in several references [16] [17], not very high due to the following fact: the impossibility to control the ionic strength, the high alkaline error in the pH measurements, the hypothesis of constant values of δ_L and δ_{LH} throughout the protonation sequence and the empirical pH calibration in D_2O media. But NMR is still the only technique for obtaining such high protonation constants besides the calorimetric method.

Signal *D* in the ^{1}H-NMR spectrum of do3a shifts to higher field to the largest extent among the four signals. Since the *multiplet D* originates from the protons *d*1 and *d*2, the protonation between pH 11 and 13 for do3a most probably occurs at N(4). The smaller shifts of the signals *A*, *B*, and *C* may be attributed either to the partial protonation of the other N-atoms or to H-bonding between the protonated N-atoms and the carboxylates or the unprotonated N-atoms. The relative intensities of the signals allows to make the following assignments: *A* to protons *a*, *B* to protons *b*, *C* to protons *c1* and *c2*.



do3a



odo3a

The other protonation constants K_2, K_3, and K_4 (*Table 1*) were determined by pH-titrations and calculated with the program TITFIT [9], while K_1 was kept constant at the value obtained from the ^{1}H-NMR titration, giving an excellent fit with $\sigma(\text{ml}) = 6.9 \cdot 10^{-4}$. For comparison, the protonation constants of dota and odo3a (= 10-oxa-1,4,7-triaza-cyclododecane-1,4,7-triacetic acid) are also shown in *Table 1*.

Table 1. *Protonation Constants of do3a, dota, and odo3a at 25°*

	$\log K_1$	$\log K_2$	$\log K_3$	$\log K_4$	Ref.
do3a[b])	11.96[a])	9.66(2)	4.23(1)	3.51(2)	This work
do3a[c])	11.59(3)	9.24(3)	4.43(7)	3.48(1)	[19]
dota[c])	12.09	9.68	4.55	4.13	[18]
odo3a[c])	11.61	7.70	4.05		[16]

[a]) NMR Measurements without control of ionic strength.
[b]) $I = 0.5$M (KNO_3).
[c]) $I = 0.1$M $(CH_3)_4N(NO_3)$.

The protonation constants of do3a are closely similar to those of dota [18], but different from those of odo3a [16]. The introduction of an O-atom does not significantly

influence K_1 and K_3, but strongly changes K_2. It was found, that when acid is added to the completely deprotonated ligand odo3a, the protonation begins at N(2). Further acidification results in a proton rearrangement from N(2) to the two N-atoms N(1), which are *trans* to each other. The third protonation step occurs at carboxylate O(4) and the fourth at carboxylate O(3) [16]. The protonation sequence of dota is such, that two N-atoms (probably *trans* to each other) are protonated first [20]; further acidification results in the preferential protonation of one of the COOH groups. Hence, in both ligands the first proton binds to a N-atom, which thus results in a similar value of K_1. The rearrangement during the second protonation to N(1) in odo3a may be the reason for its very different value of K_2 compared to dota. The similar order for the protonation constants of do3a and dota suggests that do3a exhibits the same protonation sequence as dota.

Further evidence of it is given by ^{13}C-NMR spectra (*Table 2*). The ^{13}C-NMR spectra of do3a at pH 2.1 and pH 4.0 show eight resonances *A1*, *B1*, *C1*, *D1*, *E1*, *F1*, *G1*, and *H1*. From the chemical shifts, we can assign *A1* and *B1* to the carboxylate C-atoms and *C1–H1* to the CH_2 C-atoms. The relative intensity (2:1) enables us to further assign *A1* to C-atom *f* and *B1* to C-atom *e*.

Table 2. *Chemical Shifts* [ppm] *of the* ^{13}*C-NMR Signals of do3a and [In(do3a)] at 25°*

	pH	*A1*	*B1*	*C1*	*D1*	*E1*	*F1*	*G1*	*H1*
do3a	2.1	177.36	172.57	58.49	55.93	54.39	51.69	50.44	45.04
do3a	4.0	179.15	173.10	59.03	57.86	54.67	52.34	51.31	45.57
[In(do3a)][a])	1.7	178.51	178.25	61.68	60.29	56.36	54.18	53.18	43.96
[In(do3a)][a])	4.0	178.78	178.52	61.98	60.59	56.66	54.46	53.47	44.25
[In(do3a)][b])	4.0	178.94	178.69	62.15	60.76	56.83	54.64	53.64	44.42
		179.18	173.15	59.13	57.97	54.73	52.42	51.36	45.64

[a]) $[In^{3+}]/[dota] = 1:1$.

[b]) $[In^{3+}]/[dota] = 1:2$, upper line [In(do3a)], lower line do3a resonances.

Table 2 shows that all resonances are shifted upfield upon protonation, as is generally observed for amino acids [21]. Since *A1* and *D1* are shifted to a larger extent than the other signals, we suggest that the protonation between pH 4.0 and 2.1 mainly occurs at the carboxylate C-atom *f*. In this case, *D1* can be assigned to the C-atom *b*.

NMR Spectra of [In(do3a)]. ^{13}C-NMR Spectra of both the free ligand and the In^{3+} complex using different ratios of ligand-to-metal ion concentrations were recorded at several pH values (*Table 2*). Eight peaks are observed for the In-to-ligand 1:1 mixture. In the [In(do3a)], complex the difference between the chemical shifts of the two carboxylate C-atoms becomes smaller compared to that in free do3a. These two peaks are almost superimposed, indicating that the carboxylates bind to In^{3+}, with leads to an averaged chemical surrounding. This is fully consistent with the results obtained by X-ray diffraction studies, which show that, beside the four N-atoms, also the three COO^- are coordinated to In^{3+} [22].

Comparison of the ^{13}C-NMR spectra of [In(do3a)] recorded at different pH values indicates that they do not significantly change with pH. Thus, one can conclude, that even at low pH, the In^{3+} complex is not appreciably protonated. The ^{13}C-NMR spectrum for

the In-to-ligand 1:2 mixture reveals that the signals of the free and the complexed do3a are distinguishable, thus implying that the ligand exchange is slow on the NMR time scale.

Spectrophotometric and Potentiometric Titrations of [Cu(do3a)]. The equilibria related to the complexation of do3a (L is the trianion) with Cu^{2+} are defined by *Eqns. 2–5*,

$$Cu^{2+} + L \rightleftharpoons [CuL];\ K_{CuL} \tag{2}$$

$$Cu^{2+} + LH \rightleftharpoons [CuLH];\ K_{CuLH} \tag{3}$$

$$Cu^{2+} + LH_2 \rightleftharpoons [CuLH_2];\ K_{CuLH_2} \tag{4}$$

$$Cu^{2+} + LH_3 \rightleftharpoons [CuLH_3];\ K_{CuLH_3} \tag{5}$$

whereas the protonation constants of the single species are given by *Eqn. 6*.

$$[CuLH_{i-1}] + H^+ \rightleftharpoons [CuLH_i];\ K_i \tag{6}$$

Because the protonation to $[CuLH_3]$ and $[CuLH_2]$ takes place at relatively low pH, the constants $K_{[CuLH_3]}$ and $K_{[CuLH_2]}$ (or K_3 and K_2) had to be determined by spectrophotometric titrations without control of the ionic strength. *Fig. 2* shows the absorbance at 650 nm as a function of pH.

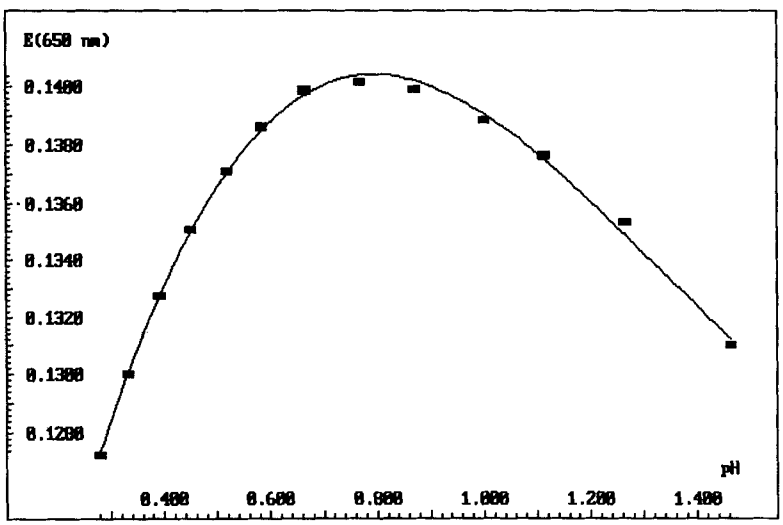


Fig. 2. *Spectrophotometric titration of the Cu^{2+} complex with do3a* ($C_M = 1.0 \cdot 10^{-3}$ M, $C_L = 1.1 \cdot 10^{-3}$ M). Absorbance at 650 nm as a function of pH.

The results obtained by fitting such curves with the program SPECFIT [11] are mean values of three titrations (*Table 3*). The reproducibility of $K_{[CuLH_3]}$ and $K_{[CuLH_2]}$ is not especially good, probably because the ionic strength could not be controlled, the acidity, at which the experiments were carried out, is relatively high, and the general difficulty in determining the stability constants of very stable complexes. We have to point out, that the model used probably is not complete. At the low pH values, at which the stability of $[CuLH_3]$ and $[CuLH_2]$ was determined, species like LH_5 and LH_6 must exist, which,

Table 3. *Stability Constants of the Cu^{2+} Complexes with do3a and dota* [18] *at 25°*

	$\log K_{[CuL]}$	$\log K_{[CuLH]}$	$\log K_{[CuLH_2]}$	$\log K_{[CuLH_3]}$	$\log K_1$	$\log K_2$	$\log K_3$
do3a	26.49(2)[b])	17.91(3)[b])	10.8(1)[a])	7.6(1)[a])	3.38(4)[b])	2.6(1)[a])	1.1(1)[a])
dota[c])	22.21	14.42	8.32		4.36	3.58	

[a]) Without control of the ionic strength. [b]) $I = 0.5$M (KNO_3). [c]) $I = 0.1$M ($(CH_3)_4N(NO_3)$).

however, we could not take into account, since we were not able to determine any protonation constants below pH 2.

The constants $K_{[CuL]}$ and $K_{[CuLH]}$ were calculated from the potentiometric titrations (*Fig. 3*) with the program TITFIT [9]. In this calculation, the values $K_{[CuLH_3]}$ and $K_{[CuLH_2]}$, obtained from the spectrophotometry, were included and kept constant.

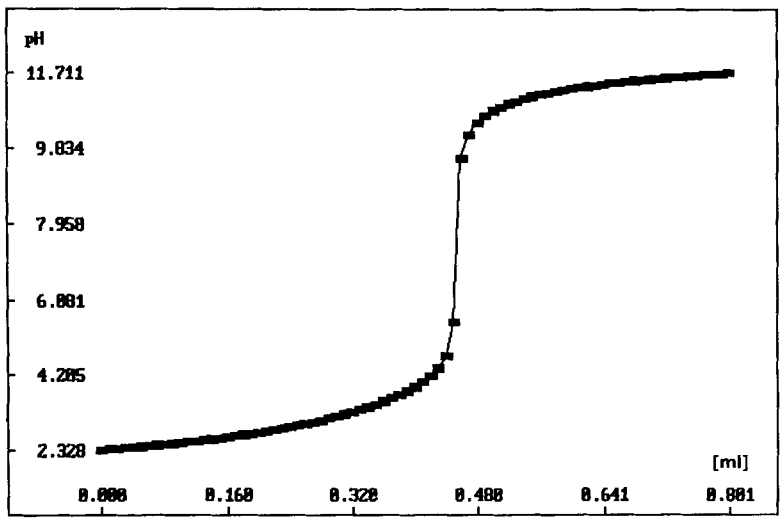


Fig. 3. *Potentiometric titration of [Cu(do3a)]* ($C_L = 2.5 \cdot 10^{-3}$M, $C_M = 2.25 \cdot 10^{-3}$M)

Table 3 shows that [Cu(do3a)] is very stable, log $K_{[CuL]}$ being about four orders of magnitude larger than that of [Cu(dota)]. This is surprising and chemically not understandable. It should, however, be noticed that, in our calculations, we have used a model containing the additional species $[CuLH_3]$, which was not considered for dota. When only the three species [CuL], [CuLH], and $[CuLH_2]$, as used by *Delgado* and *Fraústo Da Silva* for dota [18], were taken into account, and the refinement of the potentiometric measurements was done, the resulting log $K_{[CuL]}$ was 23.1, a value comparable to that found for [Cu(dota)].

Formation Kinetics of the do3a Complexes. The formation kinetics were studied at different pH values, varying the total concentration of the metal ion (C_M), while keeping the total concentration of the ligand (C_L) constant. When the ratio C_M/C_L was larger than 9.8, first-order reactions with respect to the ligand were observed regardless of the pH value. When C_M/C_L was less than 5, second-order kinetics was found.

Plots of log $k_{obs.}$ against log C_M at fixed pH give 'saturation curves' for [Y(do3a)], [Gd(do3a)], [Ga(do3a)], and [Cu(do3a)] (*Figs. 4–7*). This behavior is characteristic for the rapid formation of reaction intermediates $([MLH_i])_{in}$, which rearrange to the final product in a slow, rate-determining process [23]. So, reaction scheme (*Eqn. 7*) can be proposed, since in the pH range studied do3a is present in the three protonated forms LH_4, LH_3, and LH_2.

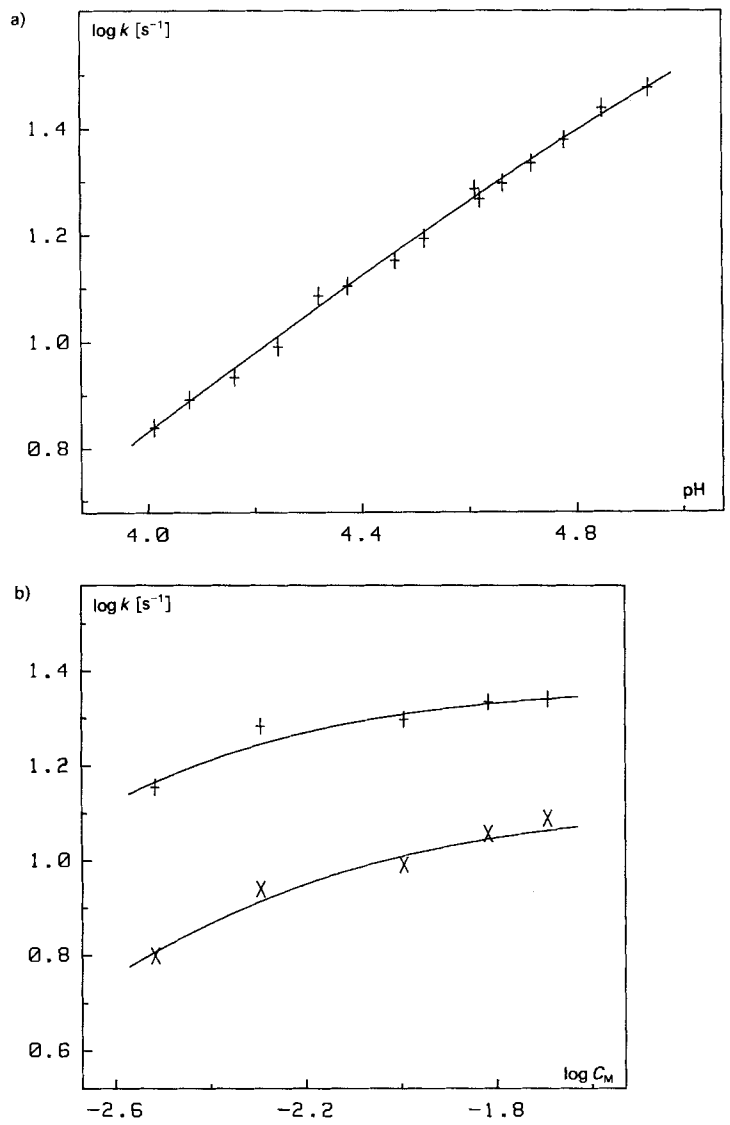


Fig. 4. *Formation of [Cu(do3a)] at 25° and 0.5 M (KNO_3) fitted with model* II. *a*) Plot of log $k_{obs.}$ *vs.* pH with $C_L = 1.0 \cdot 10^{-3}$ M and $C_M = 1.0 \cdot 10^{-2}$ M. *b*) Plot of log $k_{obs.}$ *vs.* log C_M at pH = 4.67 (+), pH = 4.24 (×), and $C_L = 1.0 \cdot 10^{-3}$ M.

$$\begin{array}{ccccc} LH_4 & & ([MLH_3])_{in} & & \\ \uparrow\downarrow & & \uparrow\downarrow K'_3 & & \\ LH_3 + M & \overset{\text{fast}}{\underset{K'_{[MLH_i]}}{\rightleftharpoons}} & ([MLH_2])_{in} & \xrightarrow[k_i]{\text{slow}} & \text{product} \\ \uparrow\downarrow & & \uparrow\downarrow K'_2 & & \\ LH_2 & & ([MLH_1])_{in} & & \end{array} \tag{7}$$

In the case of [Cu(do3a)], the pH and metal-ion-concentration dependencies were fitted either with *Eqn. 8* (model *I*), which assumes that only $([MLH])_{in}$ is reactive,

$$k_{obsd} = \frac{k_1[([MLH])_{in}]}{C_L} \tag{8}$$

or with *Eqn. 9* (model *II*), in which $([MLH])_{in}$ and $([MLH_2])_{in}$ are the reactive species,

$$k_{obsd} = \frac{k_1[([MLH])_{in}] + k_2[([MLH_2])_{in}]}{C_L} \tag{9}$$

and k_1 and k_2 are the rate constants for the intermediates $([MLH])_{in}$ and $([MLH_2])_{in}$, respectively. The stability constants of the intermediates are given by *Eqns. 10* and *11*.

$$M + LH_i \rightleftharpoons ([MLH_i])_{in} \quad K'_{[MLH_i]} \qquad i = 1,2 \tag{10}$$

$$([MLH_{n-1}])_{in} + H^+ \rightleftharpoons ([MLH_n])_{in} \quad K'_n \qquad n = 2,3 \tag{11}$$

The concentrations of the species were obtained by solving *Eqns. 12* and *13*.

$$C_L = [LH_4] + [LH_3] + [LH_2] + [([MLH])_{in}] + [([MLH_2])_{in}] + [([MLH_3])_{in}] \tag{12}$$

$$C_M = [M] + [([MLH])_{in}] + [([MLH_2])_{in}] + [([MLH_3])_{in}] \tag{13}$$

Table 4 shows that the fit of the experimental data can significantly be improved, when two reactive species are considered (model *II*). However, the standard deviations of the parameters, especially that for K'_2 and k_1, become large compared to those of model *I*. This means that the parameters are correlated among each other. Since K'_2 does not fall into the pH range studied, it is impossible to determine its value without incurring into

Table 4. *Results of the Formation Kinetics for [M(do3a)] at 25° and 0.5 M (KNO_3)*

Model	[Cu(do3a)]			[Y(do3a)]	[Gd(do3a)]		[Ga(do3a)]	
	I	*II*	*III*	*I*	*I*	*II*	*I*	*II*
$\log K_{[MLH]}$	7.82(5)	7.4(1)	7.36(4)	8.98(8)[a]	8.7(1)[a]	8.7[a]	11.2(1)[a]	11.2[a]
$\log K_{[MLH2]}$	2.74(5)[b]	2.9(1)[b]	2.9[b]	3.82(4)	4.0(1)	4.0(1)	5.0(1)	5.0(1)
$\log K_{[MLH3]}$				2.9[d]	3.2[d]	3.2[d]		
$\log K'_2$	4.58(2)	5.2(1)	5.2[c]	4.58(8)	5.0(2)	5.0[c]	3.5(1)	3.5[c]
$\log K'_3$				3.28(8)	3.4(1)	3.4(1)		
k_1 [s^{-1}]	42(3)	80(16)	80(2)	$8(1)\cdot10^{-3}$	$1.3(9)\cdot10^{-2}$	$1.31(7)\cdot10^{-2}$	$7(1)\cdot10^{-3}$	$6.8(3)\cdot10^{-3}$
k_2 [s^{-1}]		4.8(6)	4.8(4)					
$\sigma\cdot10^2$	2.7	1.7	1.6	4.2	4.8	4.8	4.0	3.9

[a]) Calculated from $K_{[MLH]}$ and K_2.
[b]) Calculated from $K_{[MLH]}$ and K'_2.
[c]) Kept constant in this calculation, to be considered as lowest limit.
[d]) Calculated from $K_{[MLH2]}$ and K'_3.

large uncertainties. On the other hand, we were not able to obtain more points at higher pH values because of $Cu(OH)_2$ precipitation. So K'_2 was kept fixed in the calculation and the value probably is only the lower limit (model *III*).

A comparison of the stability constants of the intermediates $([CuLH])_{in}$ and $([CuLH_2])_{in}$ with those of known complexes listed in *Table 5*, shows that $\log K'_{[CuLH_2]}$ is comparable to the stability constant of the acetate complex, whereas $\log K'_{[CuLH]}$ is close to the value of the glycine complex. This suggests that in $([CuLH_2])_{in}$ probably only one or two carboxylate groups are coordinated to Cu^{2+}, and that in $([CuLH])_{in}$ one carboxylate and one N-atom are bound to the metal ion. The rate constant k_1 for $([CuLH])_{in}$ is larger than k_2 for $([CuLH_2])_{in}$. Since the rate-determining step is associated with a structural rearrangement of the intermediate, the less protonated form is expected to be more reactive.

Table 5. *Stability Constants of Several Y^{3+}, Gd^{3+}, and Cu^{3+} Complexes at* I = 0.1 M

Ligand	$\log K^{M}_{[ML]}$			$\log K^{[ML]}_{[ML2]}$		
	Y^{3+}	Gd^{3+}	Cu^{2+}	Y^{3+}	Gd^{3+}	Cu^{2+}
AcOH[a])	1.97	2.16	1.89	1.63	1.60	1.20
Glycine[b])			8.07			6.90
EDDA[b])	7.78	8.13	16.2[c])	6.34	6.08	

[a]) $T = 20°$ [24]. [b]) [25]. [c]) $T = 30°$.

In the case of [Y(do3a)], [Gd(do3a)], and [Ga(do3a)], the number of protons n (per mole ligand) displaced in the complexation is known from the amount of NaOH consumed during the reaction. This can be used as an additional information, since the value of n is related to the concentrations according to *Eqn. 14*. n is a function of $K'_{[MLH_i]}$ and K'_n,

$$n = 4[LH_4] + 3[LH_3] + 2[LH_2] + [([MLH])_{in}] + 2[([MLH_2])_{in}] + 3[([MLH_3])_{in}] \quad (14)$$

whereas $k_{obs.}$ depends on $K'_{[MLH_i]}$, K'_n, and k_i. In other words, we have two functions to be fitted and the parameters must be consistent for both of them. Since $k_{obs.}$ and n have different units and uncertainties, a weighted fit must be used in this case, the weighting factors being estimated using *Eqn. 15* [26], where σ_i are the standard deviations

$$\omega_i = \frac{1/\sigma_i^2}{(1/N)\Sigma(1/\sigma_i^2)} \quad (15)$$

obtained from the independent fits of $\log k_{obs.}$ and of n. The values were usually 0.03–0.04 log units for the former and 0.07–0.15 units for the latter. N Represents the total number of experimental points. The weighted nonlinear least-squares analysis of both $\log k_{obs.}$ and n leads to the results shown in *Table 4* and the quality of the fitting can be taken from *Figs. 5–7*.

The information on n obtained from the pH-stat measurements helps to fit the experimental data and provides an additional evidence for the proposed reaction mechanism. *Table 4* shows that K'_2 is reasonably well determined for [Y(do3a)], whereas for [Gd(do3a)] and [Ga(do3a)] the standard deviations of the parameters, especially that of K'_2, are very large (model *I*). However, if K'_2 is kept fixed in the calculation, the standard deviations of the other parameters decrease (model *II*). This arises either from the

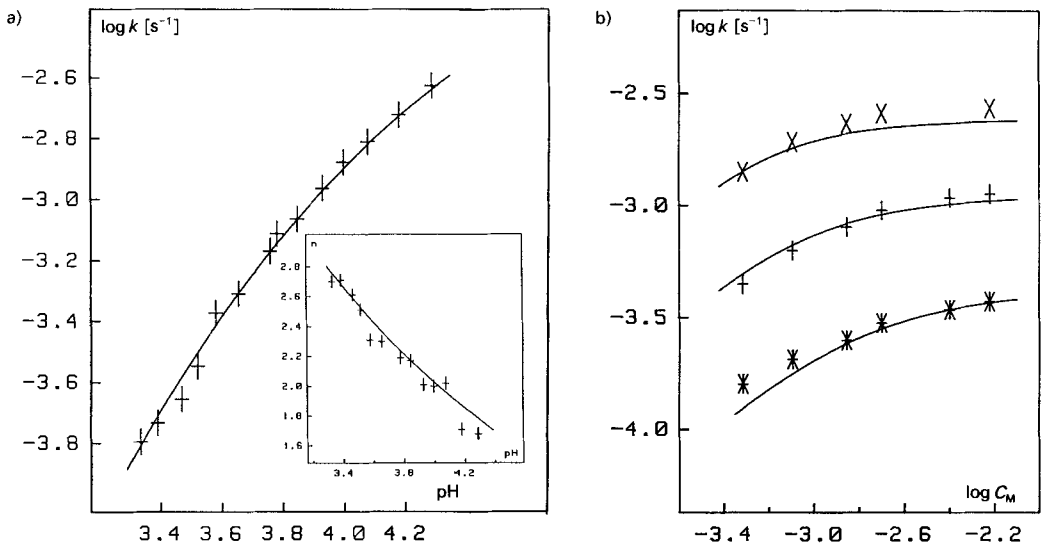


Fig. 5. *Formation of [Y(do3a)] at 25° and* I = 0.5 M *(KNO_3). a*) Plot of $\log k_{obs.}$ *vs.* pH with $C_L = 4.1 \cdot 10^{-4}$ M and $C_M = 4.0 \cdot 10^{-3}$ M (inset *n vs.* pH). *b*) Plot of $\log k_{obs.}$ *vs.* $\log C_M$ at pH 4.308 (×), pH 3.930 (+), pH 3.580 (*), and $C_L = 4.1 \cdot 10^{-4}$ M.

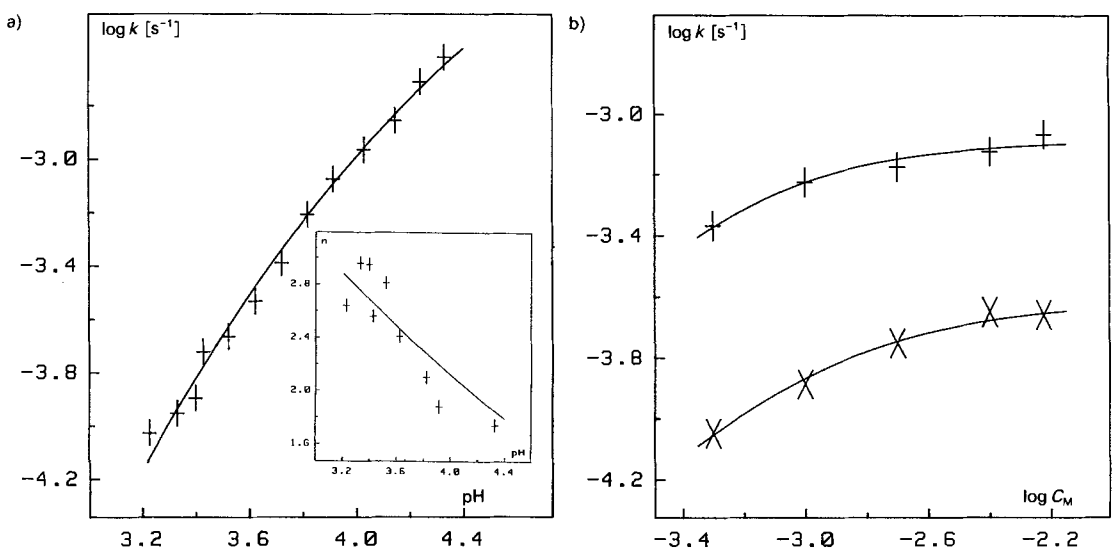


Fig. 6. *Formation of [Gd(do3a)] at 25° and* I = 0.5 M *(KNO_3). a*) Plot of $\log k_{obs.}$ *vs.* pH with $C_L = 4.1 \cdot 10^{-4}$ M and $C_M = 4.0 \cdot 10^{-3}$ M (inset *n vs.* pH). *b*) Plot of $\log k_{obs.}$ *vs.* $\log C_M$ at pH 3.492 (×), pH 3.894 (+), and $C_L = 4.1 \cdot 10^{-4}$ M.

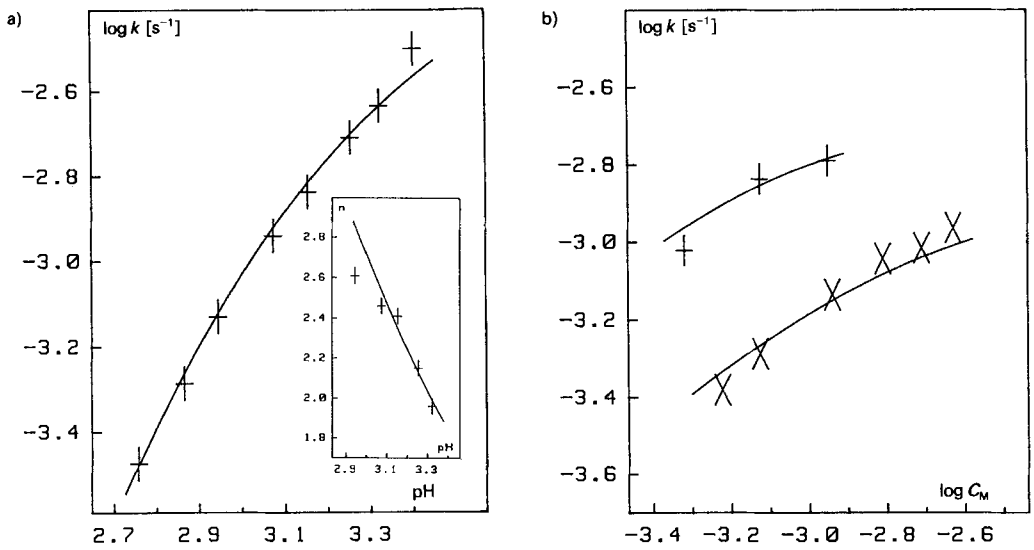


Fig. 7. *Formation of [Ga(do3a)] at 25° and* I = 0.5 M (*KNO*$_3$). *a*) Plot of $\log k_{obs.}$ *vs.* pH with $C_L = 4.1 \cdot 10^{-4}$ M and $C_M = 4.0 \cdot 10^{-3}$ M (inset *n vs.* pH). *b*) Plot of $\log k_{obs.}$ *vs.* $\log C_M$ at pH 2.865 (×), pH 3.125 (+), and $C_L = 4.1 \cdot 10^{-4}$ M.

correlation among the parameters or from the relatively few experimental points at high pH, which is due to the limitation of the pH-stat method.

The proposed reaction scheme (*Eqn. 7*) fits the experimental data of $k_{obs.}$ in all cases. As for the fitting of *n*, the situation is not always satisfactory, because data of good quality are relatively difficult to obtain, especially for [Gd(do3a)] (*Fig. 6a*). As mentioned in *Experimental*, the Gd^{3+} solution was prepared by dissolving Gd_2O_3 in an excess of HNO_3. This leads to a big pH drop upon the addition of the Gd^{3+} solution to the reaction mixture. It was, therefore, difficult to determine the exact beginning of the formation, since the reaction could only be followed after 0.1–0.25 ml of NaOH had been already added. This affects the exact determination of the amount of NaOH consumed and, therefore, also that of *n*.

As for Cu^{2+}, the comparison of the stability constants of the intermediates with those given in *Table 5* allows to suggest that, in $([MLH_2])_{in}$ and $([MLH_3])_{in}$, only one or two carboxylates are coordinated to Y^{3+} and Gd^{3+}, and that probably two O- and two N-atoms are bound in $([MLH])_{in}$. The stability constants for the corresponding Ga^{3+} complexes are not available, so the structure of its intermediates can only be suggested by analogy.

All rate constants k_1 and k_2 (*Table 4*) are much smaller than the corresponding H_2O-exchange rates [27]. This implies that the dissociative mechanism can not be operative for the formation of the complexes with do3a. The stability constants of the intermediates also indicate that several bonds are present in the intermediates. So, it is clear that the formation of the first bond can not be rate-determining, in contrast to what is mainly observed for the formation of metal complexes with open-chain ligands [28]. The steric constraints imposed by the cyclic structure of do3a make the rate-determining step occur later along the reaction pathway. The rearrangement of the ligand, which is only partially

bound in the intermediates, to a fully coordinated species, is the slow step of these reactions.

The kinetic behavior of the formation of [In(do3a)] is different from those of the other metal ions. Two first-order reactions are needed to fit the experimental data. The rate constants of the faster reactions are irregularly scattered over the pH range studied, probably because the reaction is too fast to be determined under the present conditions. The formation kinetics of [In(do3a)] has not been studied in detail, because of the fewer available points limited by the pH-stat method. For the slower reaction, log $k_{obs.}$ is independent of log C_M under the present conditions. Linear-least-square analysis of the plot of log $k_{obs.}$ *vs.* pH for the slower reaction results in a slope of 2.5 and an intercept of −9.5 (*Fig. 8*).

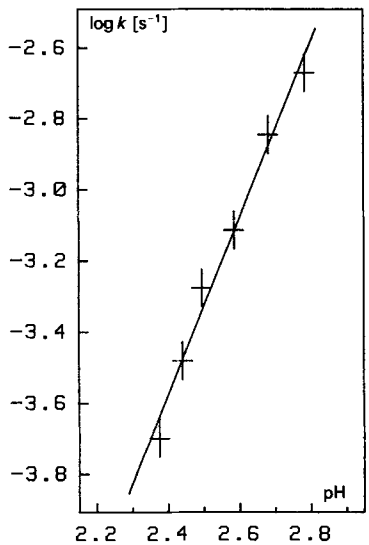


Fig. 8. *Plot of log* $k_{obs.}$ vs. *pH for the Formation of [In(do3a)] at 25°.* $I = 0.5$M (KNO_3), $C_L = 4.1 \cdot 10^{-4}$M, and $C_M = 8.1 \cdot 10^{-4}$M.

Since for the slower step $k_{obs.}$ is independent of C_M, the intermediates, which are formed *via* a rapid preequilibrium, must be very stable. Therefore, the reaction can be described by *Eqn. 16*, which assumes that the intermediate is a protonated species $([InLH_i])_{in}$ and the final product is [InL].

$$([InLH_i])_{in} \xrightarrow{k} [InL] + nH^+ \tag{16}$$

It is concluded from the slope of 2.5 for log k_{obsd} *vs.* pH that 2–3 protons are probably involved in the intermediate. This formation is the most rapid one of all reactions of trivalent metal ions with do3a in this study.

Dissociation Kinetics of [Y(do3a)], [Gd(do3a)], and [Cu(do3a)]. The experimental data fit pseudo-first-order kinetics with respect to the complex in all cases. Plots of the pseudo-first-order rate constant log $k_{obs.}$ *vs.* pH are shown in *Figs. 9* and *10*. The do3a complexes of the trivalent metal ions and of Cu^{2+} are all kinetically stable even in slightly acidic solution. So the dissociation can only be measured at pH < 2.

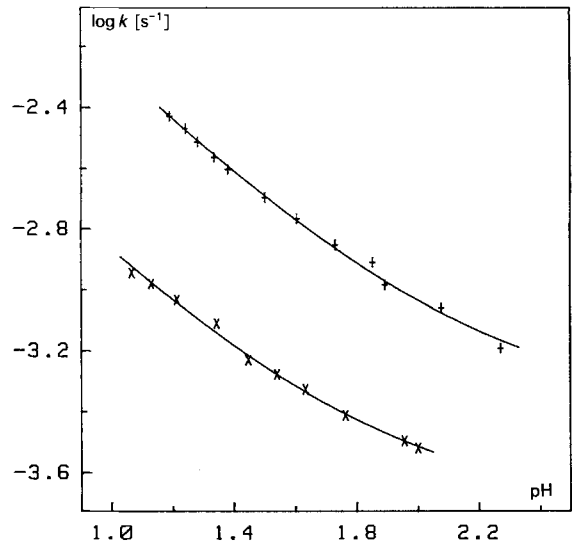


Fig. 9. *Plot of log* $k_{obs.}$ *vs. pH for the Dissociation of [Y(do3a)] (+) and [Gd(do3a)] (×) at 25°. I* = 0.5M (KNO_3) and $C_{Complex} = 2.0 \cdot 10^{-5}$ M.

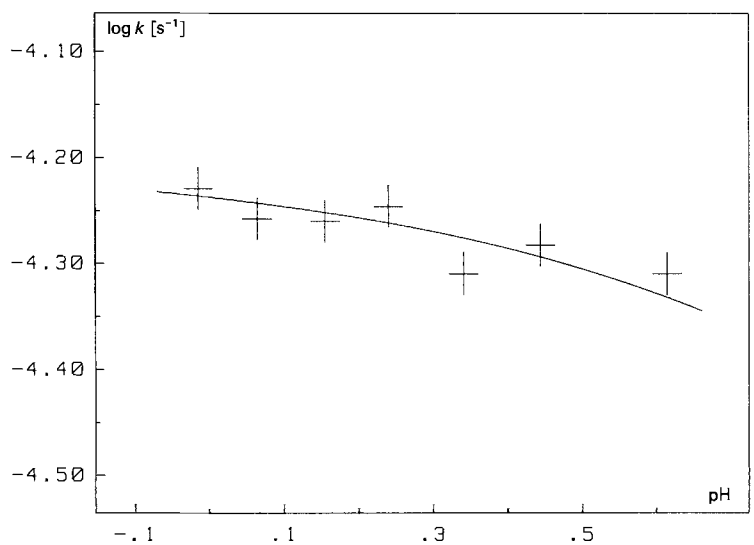


Fig. 10. *Plot of log* $k_{obs.}$ *vs. pH for the Dissociation of [Cu(do3a)] at 25°. I* = 1.0M (KNO_3) and $C_{Complex} = 1.0 \cdot 10^{-3}$ M.

The measurements presented in *Fig. 9* can be fitted with *Eqn. 17*, where k_0 and k_1 were resolved

$$k_{obs.} = k_0 + k_1 \cdot [H^+] \tag{17}$$

by least-squares analysis of the data with standard deviation $\sigma(\log k_{obs}) < 1.7 \cdot 10^{-2}$ (*Table 6*).

Table 6. *Rate Constants for the Dissociation of the [Y(do3a)] and [Gd(do3a)] Complexes at 25° and I = 0.5 M (KNO_3)*

	$k_0 \cdot 10^4$ [s^{-1}]	$k_1 \cdot 10^2$ [$M^{-1}s^{-1}$]
[Y(do3a)]	4.1(2)	5.2(1)
[Gd(do3a)]	1.90(8), 4.4 [19]	1.17(3), 2.6 [19][a])

[a]) Calculated from K_1 and k_2 of [19].

The spectrophotometric and potentiometric titrations indicate that $[CuLH_2]$ and $[CuLH_3]$ are the main species under the present conditions. Thus, reaction scheme (*Eqn. 18*) can be proposed, in which the rapid preequilibrium $[CuLH_2]$ and $[CuLH_3]$ is followed by

$$[CuLH_2] + H^+ \underset{\text{fast}}{\overset{K_3}{\rightleftharpoons}} [CuLH_3] \underset{\text{slow}}{\overset{k_1}{\rightarrow}} Cu^{2+} + LH_3 \tag{18}$$

a pH-independent dissociation of $[CuLH_3]$, which is the rate-determining step. This leads to *Eqn. 19*, where $C_{complex}$ is the total concentration of the complex (*Eqn. 20*).

$$k_{obs.}\, C_{complex} = k \cdot [[CuLH_3]] \tag{19}$$

$$C_{complex} = [[CuLH_2]] + [[CuLH_3]] \tag{20}$$

If $[[CuLH_3]]$ is calculated from in *Eqn. 21*, using $K_3 = 11.75$ as determined by

$$[[CuLH_3]] = K_3 \cdot [[CuLH_2]] \cdot [H^+] \tag{21}$$

spectrophotometric titrations, $C_{complex}$ is then given by the *Eqn. 22*.

$$C_{complex} = [[CuLH_2]] \cdot (1 + K_3 \cdot [H^+]) \tag{22}$$

The final *Eqn. 23* is obtained by substitution of *Eqns. 22* and *21* into *Eqn. 19*.

$$k_{obs.} = \frac{k \cdot K_3}{1 + K_3 \cdot [H^+]} \tag{23}$$

Least-square analysis of $\log k_{obs.}$ *vs.* pH gave $k = 6.3(1) \cdot 10^{-5}$ s^{-1} with the standard deviation $\sigma(\log k_{obs.}) = 2 \cdot 10^{-2}$. The slight pH dependence of $k_{obs.}$, as shown in *Fig. 10*, comes from the fact that both $[CuLH_3]$ and $[CuLH_2]$ are present, and the equilibrium between these two species is pH-dependent.

The dissociation of [CuL] thus goes through the rapid formation of $[CuLH_3]$, which then slowly dissociates to Cu^{2+} and the protonated ligand in a pH independent process. Since the intermediate $[CuLH_3]$ can be studied spectroscopically, some information on its structure can be derived. In the case of 1,4,7,10-tetraazacyclododecane, the absoption maximum of the Cu^{2+} complex, in which four N-atoms are coordinated to the metal ion, is 590 nm [29]. If in the case of do3a, the three carboxylates in $[CuLH_3]$ were protonated, the λ_{max} for $[CuLH_3]$ should be close to 590 nm. However, the spectrophotometric titration gives $\lambda_{max} = 651$ nm. Therefore, we suggest, that besides the carboxylates at least one N-atom might also be protonated in $[CuLH_3]$, and that the macrocycle is already partially dissociated from the metal ion. Probably because of the relatively high charge of +2 in $[CuLH_3]$, no additional interaction with a proton takes place in the final dissociation.

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